Recipient (P678-54) and donor (G43:BW6169) strains were grown overnight in 10 mL of LB media (10 g NaCl, 10 g select peptone 140, and 5 g yeast extract in one liter ddH20). The samples were centrifuged and then concentrated in about 0.2 mL of LB media. The concentrated samples were combined and incubated with slow rotation for 30 minutes at 30°C, and were then plated on LB agar plates that contained streptomycin (50 μg/mL) and tetracycline (50 μg/mL). (Ampicillin, streptomycin, tetracycline, and all other chemicals were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated.) Recipient cells were resistant to streptomycin and donor cells were resistant to tetracycline; only conjugates, which contained both resistance genes, were able to grow on the LB agar plates that contained streptomycin (50 μg/mL) and tetracycline (50 μg/mL).

Putative conjugates were screened for Lambda phage sensitivity using a cross streak technique, in which putative colonies were cross-streaked on an LB agarose plate (streptomycin, 50 μg/mL, and tetracycline, 50 μg/mL) that had been streaked with live Lambda phage. The streaked conjugate colonies were streaked perpendicular to the Lambda phage streak; if a conjugate was sensitive to Lambda phage infection then, upon contact with the Lambda phage streak, there was cell lysis and thus less or no bacterial growth. Thus, in the case of conjugates that were sensitive to Lambda phage, there was deceased bacterial growth "downstreak" from the phage streak.

The conjugate E. coli that were found to be sensitive to Lambda phage infection were then used to create Lambda lysogens. Lysogenization is a process during which Lambda phage incorporates its genome, including exogenous genes added thereto, into a specific site on the chromosome of its E. coli host cell.

The DE3 gene, which is present in the genome of the Lamda phage used to create lysogens, encodes RNA polymerase from bacteriophage T7. Lysogenation was carried out using the DE3-Lysogenation kit (Novagen, Madison, WI) essentially according to the manufacturer's instructions. A T7 polymerase dependent tester phage was used to confirm the presence and expression of the DE3 gene on the bacterial chromosome. The T7-dependent tester phage can only form plaques on a baterial known in the presence of T7 polymerase. The phage uses a T7 promoter for expression of its essential genes. Therefore in a plaque-forming assay only cells which express T7 polymerase can be lysed by the tester phage and only these cells will allow for the formation of plaques. As is described in more detail herein, episomal expression elements that are used in minicells may be designed such

that transcription and translation of a cloned gene is driven by T7 RNA polymerase by utilizing expression sequences specific for the T7 RNA polymerase.

EXAMPLE 2: CLONING OF RAT EDG-1 INTO THE PCAL-C EXPRESSION VECTOR

Materials

Taq Polymerase, PCR Buffers, and PCR reagents were purchased from Roche Molecular Biochemicals (Indianapolis, IN). All restriction enzymes were purchased from Gibco BRL (Grand Island, NY) and Stratagene (La Jolla, CA). QIAprep mini and maxi kits, PCR purification Kits, RNeasy miniprep kits, and the One Step RT-PCR Kit were purchased from QIAGEN (Valencia, CA). The Geneclean Kit was purchased from BIO 101 (Carlsbad, CA). IPTG (isopropy-beta-D-thiogalactopyranoside), T4 DNA Ligase, LB Media components and agarose were purchased from Gibco BRL. The pCAL-c prokaryote expression vector and competent cells were purchased from Stratagene.

The pCAL-c expression vector has a structure in which an ORF may be operably linked to a high-level (but T7 RNA polymerase dependent) promoter, sequences that bind the E. coli Lac repressor, and the strong T7 gene 10 ribosome-binding site (RBS). The LacI repressor is also encoded by an expressed from te pCAL-c vector. As long as it is bound to its recognition sequences in the pCAL-c expression element, the lac repressor blocks transcription from the T7 promoter. When an inducing agent, such as IPTG is added, the lac repressor is released from its binding sites and transcription proceeds from the T7 protmoter, provided the T7 RNA polymerase is present. After induction, the cloned and expressed protein may constitute the majority of newley expressed cellular proteins due to the efficient transcription and translation processes of the system.

Amplification

The first step in cloning rat Edg-1 (rEDG-1) into an expression vector was to design primers for amplification via PCR (polymerase chain reaction). PCR primers were designed using the rat Edg-1 sequence (Nakajima et al., Biophy, J. 78:319A, 2000) in such a manner that they contained either sites for NheI (GCTAGC) or BamHI (GGATCC) on their five prime ends. The upstream primer had the sequence of SEQ ID NO:31. The three prime downstream primer (SEQ ID NO:32) also contained a stop codon, as the pCAL-c vector contains a Calmodulin Binding Protein (CBP) "tag" at its carboxyl terminus which was not

intended to be incorporated into the rat Edg-1 polypeptide in this expression construct. The primer and resulting PCR products were designed so that the five prime end of the rat Edg-1 ORF was in frame with the methionine start codon found in the pCAL-c vector.

OLIGONUCLEOTIDE PRIMER SEQUENCES FOR CLONING INTO PCAL-C:

Edg1/pCAL-c construct primers:

Upstream primer (SEQ ID NO:31)

5'-AATTGCTAGCTCCACCAGCATCCCAGTGGTTA-3'

Downstream primer (SEQ ID NO:32)

5'-AATTGGATCCTTAAGAAGAAGAATTGACGTTT-3'

Edg1/CBP fusion construct primers:

Upstream primer (SEQ ID NO:31)

5'-AATTGCTAGCTCCACCAGCATCCCAGTGGTTA-3'

Downstream primer (SEQ ID NO:33)

5'-AATTGGATCCAGAAGAAGAATTGACGTTTCCA-3'

Edg1/His6 construct primers:

Upstream primer (SEQ ID NO:31)

5'-AATTGCTAGCTCCACCAGCATCCCAGTGGTTA-3'

Downstream primer (SEQ ID NO:34)

5'-
AATTGGATCCTTAATGATGATGATGATGATGAGAAGAAGAATTGACGTTTCC-3'

Edg3/rtPCR primers:

Upstream primer (SEQ ID NO:35)

5'-TTATGGCAACCACGCACGCGCAGG-3'

Downstream primer (SEQ ID NO:36)

5'-AGACCGTCACTTGCAGAGGAC-3'

Edg3/pCAL-c construct primers:

Upstream primer (SEQ ID NO:37)

5'-AATTGCTAGCACGCACGCGCAGGGGCACCCGC-3'

Downstream primer (SEQ ID NO:38)

5'-AATTGGTACCTCACTTGCAGAGGACCCCATTCTG-3'

Edg3/His6 construct primers:

Upstream primer (SEQ ID NO:39)

5'-AATTGCTAGCACGCACGCGCAGGGGCACCCGC-3'

Downstream primer (SEQ ID NO:16)

5'-AATTGGTACCTCAATGATGATGATGATGATGCTTGCAGAGGACCCCATTCTG-3'

GFP/pCAL-c construct primers:

Upstream primer (SEQ ID NO:40)

5'-GGTCGCCACCATGGTGAGCAA-3'

Downstream primer (SEQ ID NO:41)

5'-TTAAGGATCCTTACTTGTACAGCTCGTCCAT-3'

GFP/CBP construct primers:

Upstream primer (SEQ ID NO:42)

5'-GGTCGCCACCATGGTGAGCAA-3'

Downstream primer (SEQ ID NO:43)

5'-TTAAGGATCCCTTGTACAGCTCGTCCATGCC-3'

Notes:

Restriction endonuclease sites are underlined

Stop codons are double underlined

The primers were used to amplify the rEdg-1 DNA ORF using the polymerase chain reaction (PCR). The template used for amplification was mRNA isolated from rat muscle tissue using the RNeasy Miniprep Kit (Qiagen) and was carried out essentially according to the manufacturer's protocol. Both the rtPCR and PCR amplification steps were carried out in a single reaction using the One Step RT-PCR Kit (Qiagen). The resulting rat Edg-1 PCR fragment was purified using the PCR Purification Kit (Qiagen). The amplified double stranded rEdg-1 DNA sequence contained the NheI site at the 5-prime end and the BamHI site at the 3-prime end. This amplified rEdg-1 fragment was used for cloning into the pCAL-c expression vector.

The pCAL-c expression vector contains NcoI, NheI, and BamHI restriction sites in its multiple cloning site. In order to insert rEdg-1-encoding sequence into the expression vector, the rEdg-1 PCR fragment and the pCAL-c expression vector were digested with NheI and BamHI restriction enzymes for one hour at 37°C. The reaction mixture for the digestion step consisted of 1 μg of DNA, 1x restriction buffer, and 1 μL of each enzyme. The reaction mixture was brought to a final volume of 20 μL with ddH_2O (dd, double distilled). After 45 minutes, 1 μL of Calf Intestine Alkaline Phosphatase (CIAP) was added to the pCAL-c reaction mixture in order to remove the terminal phosphates from the digested plasmid DNA.

The reactions were incubated for an additional 15 minutes at 37°C. The digested DNA samples were then run on a 1% TAE (Tris-acetate/EDTA electrophoresis buffer) agarose gel at 130 volts for 45 minutes. The bands were visualized with UV light after the gel was stained with ethidium bromide.

The appropriate bands were cut out of the gel for purification using the Geneclean Kit (BIO101). The Purified DNA fragments were then quantified on a 1% TAE agarose gel. For the ligation reaction, ratios of insert to vector of 6:1 and 3:1 were used. A negative control comprising vector only was also included in the ligation reactions. The reaction mixtures contained insert and vector DNA, 4 μL Ligase buffer, and 2 μL Ligase. The reaction was brought up to a final volume of 20 μL with ddH_2O. The ligation was carried out at room temperature for about 2 hours. Ten (10) μL of the ligation reaction mixture was used for subsequent transformation steps.

Ligated DNA was introduced into Epicurian Coli XL1-Blue competent cells using the heat shock transformation technique as follows. The ligation mixture was added to 100 μL of competent cells, placed on ice, and was incubated for about 30 minutes. The cells were then heat shocked at 37°C for 1 minute and put back on ice for 2 minutes. Following heat shock, 950 μL of room temperature LB media was added to the cells and the cells were shaken at 37°C for 1 hour. Following the 1-hour agitation the cells were pelleted for one minute at 12000 rpm in a Eppendorf 5417C microcentrifuge. The supernatant was carefully poured off so that about 200 μL remained. The cells were then resuspended in the remaining LB media and spread on 100x15 mm LB agarose plates containing 50 μg/mL ampicilin. The plates were incubated overnight at 37°C. Colonies were counted the following day, and the ratio of colonies between the negative control and the ligated samples was determined. A high ratio of the number of colonies when the ligation mixture was used to transform cells, as contrasted to the number of negative control colonies indicated that the cloning was successful. Transformed colonies were identified, isolated, and grown overnight in LB media in the presence of ampicillin. The resulting bacterial populations were screened for the presence of the Edg-1-pCAL-c expression construct.

Plasmid DNA was isolated from the cells using the QIAprep Spin Miniprep Kit (Qiagen). Isolated Edg-1-pCAL-c constructs were screened using the restriction enzyme ApaI, which digests the Edg-1-pCAL-c construct at two different sites: one in the Edg-1 coding sequence and one in the pCAL-c vector itself. The plasmid preparations were digested

with ApaI electrophoresed on a 1% TAE agarose gel and visualized using uv light and ethidium bromide staining. The predicted sizes of the expected DNA fragments were 2065 bp and 4913 bp. As shown in Figure 3, bands of the predicted size were present on the gel. The entire Edg-1-pCAL-c construct was sequenced in order to confirm its structure. This expression construct, a pCAL-c derivative that contains the rat Edg-1 ORF operably linked to a T7 promoter and lac repressor binding sites, is designated "prEDG-1" herein.

EXAMPLE 3: CONSTRUCTION OF RAT EDG-1-CBP FUSION PROTEIN

In order to detect rat Edg-1 protein expression, rEdg-1 coding sequences were cloned into the pCAL-c vector in frame with a CBP fusion tag. The cloning strategy for the rEdg-1-CBP construct was performed essentially as described for the Edg-1-pCAL-c construct with the following differences. The PCR primers (SEQ ID NOS:3 and 5) were as described for the Edg-1-pCAL-c cloning except for the omission of the stop codon in the downstream primer (SEQ ID NO:33). The removal of the stop codon is required for the construction of the Edg-1-CBP fusion protein. The pCAL-c vector is designed so that, when the BamHI site is used for insertional cloning, and no stop codon is present in an ORF inserted into the pCAL-c expression vector the cloned ORF will be in-frame with the CBP fusion tag. Because the three prime downstream primer did not contain a stop codon, a CBP fusion tag could be cloned in-frame with the Edg-1 ORF. Other cloning steps were performed essentially as described before. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding a rat Edg-1-CBP fusion protein operably linked to a T7 promoter and lac repressor binding sites, is designated "prEDG-1-CBP" herein.

EXAMPLE 4: CLONING OF A HIS-TAGGED RAT EDG-1 INTO PCAL-C EXPRESSION VECTOR

The rEdg-1 protein was manipulated to generate a fusion protein having a 6xHis tag at its carboxyl terminus. A "6xHis tag" or "His tag" is an amino acid sequence consisting of six contiguous histidine residues that can be used as an epitope for the binding of anti-6xHis antibodies, or as ligand for binding nickel atoms. The His-tagged rEdg-1 fusion protein is used to detect rEdg-1 protein expression in the minicell expression system environment.

The rEdg-1-6xHis construct was cloned using the strategy described above for the construction of the rEdg-1-pCAL-c expression construct (prEDG-1), with the upstream primer having the sequence of SEQ ID NO:3, but with the exception that the three prime

downstream primer (SEQ ID NO:34) was designed to contain six histidine codons followed by a stop codon. The 18 base pair 6xHis tag was incorporated into the carboxyl terminus of the Edg-1 protein as expressed from the pCAL-c vector. Subsequent cloning procedures (PCR, restriction digest, gel purification, ligation, transformation, etc.) were performed as described previously for the Edg-1-pCAL-c construct (prEDG-1). The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding a carboxy-terminal His-tagged rat Edg-1-CBP fusion protein operably linked to a T7 promoter and lac repressor binding sites, is designated "prEDG-1-6xHis" herein.

EXAMPLE 5: AMPLIFICATION AND CLONING OF RAT EDG-3 SEQUENCES

The Edg-3 full length coding sequence was amplified via PCR from rat skeletal muscle mRNA using primers (SEQ ID NOS:35 and 36) designed from the known mouse sequence (Genbank accession NM_010101). The mRNA used as a template for the amplification reaction was isolated using the RNeasy Miniprep Kit (Qiagen). Both the rtPCR and PCR amplification steps were carried out in a single reaction using the One Step RT-PCR Kit (Qiagen). The rEdg-3 PCR products were visualized with UV after electrophoresis in 1% TAE agarose gels and ethidium bromide staining.

The predicted size of the amplified PCR products is 1145 base pairs. An appropriately-sized DNA band was isolated from the TAE gel and purified using the Geneclean Kit (BIO101). The purified band was ligated to the pCR3.1 vector using the TA-cloning kit (Invitrogen). Other cloning steps were carried out as described previously for the cloning of the rEdg-1-pCAL-c construct (prEDG-1)with the exception that the samples were screened using the EcoRI restriction enzyme. The expected sizes of the digested bands were 1145 base pairs and 5060 base pairs. Positive clones were analyzed by automated sequencing. The nucleotide sequences were analyzed using BLAST searches from the NCBI web site (www.ncbi.nlm.nih.gov/). The predicted full length rat Edg-3 amino acid sequence was assembled from the nucleotide sequencing data using in silico translation. The pCR3.1 vector comprising the rat Edg-3 ORF is designated "pCR-rEDG-3" herein.

EXAMPLE 6: CLONING OF RAT EDG-3 CODING SEQUENCES INTO THE PCAL-C EXPRESSION VECTOR

In order to express it in the minicell expression system, the rat Edg-3 ORF was cloned into the pCAL-c expression vector. The cloning strategy used was as described above for the cloning of the rat Edg-1 gene into the pCAL-c vector with the following exceptions.

The primers used for PCR amplification were designed from the rat Edg-3 sequence and contained sites for the restriction enzymes NheI and KpnI (GGTACC). The NheI site was added to the five prime upstream primer (SEQ ID NO:37) and the KpnI site was added to the three prime downstream primer; SEQ ID NO:38). The NheI and KpnI restriction enzymes were used for the digestion reaction. The reaction mixture for the digestion step consisted of 1 μg of DNA, 1x restriction buffer (provided with the enzyme), and 1 μL of each enzyme. Plasmid preparations were screened by digestion with NheI and KpnI. The digested plasmid DNA was electrophosesed on a TAE agarose gel and visualized by UV after staining with ethidium bromide. The resultant band sizes were predicted to be 1145 base pairs and 5782 base pairs. The positive plasmid clones were analyzed with automated sequencing. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding a rat Edg-3 protein operably linked to a T7 promoter and lac repressor binding sites, is designated "pEDG-3" herein.

EXAMPLE 7: CLONING OF A HIS-TAGGED RAT EDG-3 INTO THE PCAL-C EXPRESSION VECTOR

In order to detect expression of the rat Edg-3 protein in the minicell expression system, the rat Edg-3 coding sequence was manipulated so as to contain a 6xHis tag at the carboxyl terminus of the protein. The cloning strategy used to create this construct was essentially the same as described above for the rEdg-3-pCAL-c (prEDG-3) construct cloning, with the upstream primer having the sequence of SEQ ID NO:37, with the exception that the three-prime downstream primer (SEQ ID NO:18) was designed to contain a 6xHis coding sequence followed by a stop codon, which allowed for the incorporation of the 6xHis amino acid sequence onto the carboxyl terminus of the Edg-3 receptor protein. Other cloning and screening steps were performed as described above. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding a carboxy-terminal His-tagged rat Edg-3 fusion protein operably linked to a T7 promoter and lac repressor binding sites, is designated "prEDG-3-6xHis" herein.

EXAMPLE 8: GFP CLONING INTO PCAL-C EXPRESSION CONSTRUCT

Cloning of GFP-encoding nucleotide sequences into the pCAL-c vector was performed in order to produce an expression construct having a reporter gene that can be used to detect protein expression (GFP, green flourescent protein). The cloning strategy used was essentially the same as the cloning strategy described above with the following exceptions. The template used for PCR amplification was the peGFP plasmid "construct"

(GFP construct sold by Clontech). The primers used for amplification were designed from the GFP coding sequence and contained sites for the restriction enzymes NcoI and BamHI. The NcoI site was added to the five prime upstream primer (SEQ ID NO:40) and the BamHI site was added to the three prime downstream primer; see SEQ ID NO:41) The NcoI and BamHI restriction enzymes were used for the digestion reaction. The reaction mixture for the digestion step consisted of 1 μg of DNA, 1x restriction buffer (provided with the enzyme), and 1 μL of each enzyme. The screening of the plasmid preparations was carried out using NcoI and BamHI. Digested plasmid preparations were electrophoresed and visualized on TAE agarose gels with UV after staining with ethidium bromide. Restriction products having the predicted sizes of 797 and 5782 base pairs were seen. Positive plasmid clones were sequenced using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF encoding a rEdg-3-GFP fusion protein operably linked to a T7 promoter and lac repressor binding sites, is designated "prEDG-3-GFP" herein.

EXAMPLE 9: DESIGN CONSTRUCTION OF CONTROL EXPRESSION ELEMENTS

Control expression elements used to detect and quantify expression of proteins in minicells were preposed. These controls direct the expression of detectable proteins. An expression element used as positive control is pPTC12, which is supplied with the pCAL-c expression vector from Stratagene. This construct contains an ORF encoding a fusion protein comprising beta-galactosidase linked to CBP. Induction of expression of pTC12 should result in the production of a protein of about 120 kD, and this protein is detected via its enzymatic activity or by using antibodies directed to epitopes on the beta-galactosidase or CBP polypeptide.

A GFP fusion construct was created and used as a positive control for the CBP detection kit. This construct was a positive control for induction of protein expression in the minicell expression system. The cloning strategy used to create the construct was essentially the same as that used for the cloning of the GFP into the pCAL-c expression vector, with the exception that the three prime downstream primer did not contain a stop codon; this allowed for the in frame incorporation of the CBP fusion tag to the GFP protein. The upstream primer had the sequence of SEQ ID NO:42, and the downstream primer had the sequence SEQ ID NO:43. The nucleotide sequence of the expression element was confirmed using an automated sequencer. The resulting plasmid, a pCAL-c derivative that comprises an ORF

encoding GFP operably linked to a T7 promoter and lac repressor binding sites, is designated "pGFP-CBP" herein.

EXAMPLE 10: INTRODUCTION OF PCAL-C EXPRESSION CONSTRUCTS INTO THE MC-T7 ESCHERICHIA COLI STRAIN

The MC-T7 E. coli strain was made competent using the $CaCl_2$ technique. In brief, cells were grown in 40 mL LB medium to an OD_{600} of 0.6 to 0.8, and then centrifuged at 8000 rpm (7,700 g) for 5 min at 4°C. The pellet was resuspended in 20 mL of cold $CaCl_2$ and left on ice for five minutes. The cells were then centrifuged at 8000 rpm (7,700 g) for 5 min at 4°C. The cell pellet was resuspended in 1 mL of cold $CaCl_2$ and incubated on ice for 30 min. Following this incubation 1 mL of 25% glycerol was added to the cells and they were distributed and frozen in 200 µL aliquots. Liquid nitrogen was used to freeze the cells. These cells subsequently then used for the transformation of expression constructs.

EXAMPLE 11: PREPARATION OF MINICELLS

To some degree, the preparation of minicells varied according to the type of expression approach that is used. In general, there are two such approaches, although it should be noted from the outset that these approaches are neither limiting nor mutually exclusive. One approach is designed to isolate minicells that already contain an expressed therapeutic protein or nucleic acid. Another approach is designed to isolate minicells that will express the protein or nucleic acid in the minicell following isolation.

E. coli are inoculated into bacterial growth media (e.g., Luria broth) and grown overnight. After this, the overall protocol varies with regards to methods of induction of expression. The minicell producing cultures used to express protein post isolation are diluted and grown to the desired OD_{600} or OD450, typically in the log growth phase of bacterial cultures. The cultures are then induced with IPTG and then isolated. The IPTG concentration and exposure depended on which construct was being used, but was usually about 500 µM final for a short time, typically about 4 hours. This treatment results in the production of the T7 polymerase, which is under control of the LacUVR5 promoter, which is repressed by the LacI repressor protein. IPTG relieves the LacI repression and thus induces expression from the LacUVR5 promoter which controls expression of the T7 polymerase from the chromosome. This promoter is "leaky" that is, there is always a basal level of T7 polymerase which can be selected for or against so that the induction before isolation is not required. (This induction step is not required if a non-T7 expression system is used, as the

reason for this step is to express the T7 RNA polymerase in the minicell-producing cells so that the polymerase and molecules segregate with the minicell.)

The E. coli cultures that produce minicells containing a therapeutic protein or nucleic acid have different induction protocols. The overnight cultures are diluted as described above;. however, in the case of proteins that are not toxic to the parent cells, this time the media used for dilution already contains IPTG. The cultures are then grown to mid-log growth and minicells are isolated. These cultures produce the therapeutic protein or nucleic acid as they grow, and the minicells derived therefrom contain the therapeutic protein or nucleic acid.

Altenatively or additionally, IPTG is added and expression is induced after the isolation of minicells. In the case of non-toxic proteins or nucleic acids that are expressed from expression elements in minicells, this treatment enhances production of the eposimally encoded gene product. In the case of toxic gene products induction post-isolation is preferred.

EXAMPLE 12: MINICELL ISOLATION

Minicells were isolated from the minicell producing MC-T7 strain of E. coli using centrifugation techniques. The protocol that was used is essentially that of Jannatipour et al. (Translocation of Vibrio Harveyi N,N'-Diacetylchitobiase to the Outer Membrane of Escherichia Coli, J. Bacteriol. 169: 3785-3791, 1987) and Matsumura et al. (Synthesis of Mot and Che Products of Escherichia coli Programmed by Hybrid ColE1 Plasmids in Minicells, J. Bacteriol. 132:996-1002, 1977).

In brief, MC-T7 cells were grown overnight at 37°C in 2 to 3 mL of LB media containing ampicillin (50 μg/mL), streptomycin (50 μg/mL), and tetracycline (50 μg/mL) (ampicillin was used only when growing MC-T7 cells containing a pCAL-c expression construct). The cells were diluted 1:100 in a total volume of 100 to 200 mL LB media with antibiotics, and grown at 37°C until they reached an OD_{600} of 0.4 to 0.6, which is roughly beginning of the log growth phase for the MC-T7 E. coli. During this incubation the remainder of the overnight culture was screened for the presence of the correct expression construct using the techniques described above. When the cultures reached the appropriate OD_{600} they were transferred to 250 mL GS3 centrifuge bottles and centrifuged (Beckman

centrifuge) at 4500 rpm (3,500 g) for 5 min. At this point the supernatant contains mostly minicells, although a few relatively small whole cells may be present.

The supernatant was transferred to a clean 250 mL GS3 centrifuge bottle and centrifuged at 8000 rpm (11,300 g) for 10 min. The pellet was resuspended in 2 mL of 1x BSG (10x BSG: 85 g NaCl, 3 g KH_2PO_4, 6 g Na_2HPO_4, and 1 g gelatin in 1 L ddH_2O) and layered onto a 32 mL 5 to 20% continuous sucrose gradient. The sucrose gradient was made with sucrose dissolved in 1x BSG.

The sucrose gradient was then loaded in a Beckman SW24 rotor and centrifuged in a Beckman Ultracentrifuge at 4500 rpm (9,000 g) for 14 min. Following ultracentrifugation a single diffuse band of minicells was present. The top two thirds of this band was aspirated using a 10 mL pipette and transferred to a 30 mL Oakridge tube containing 10 mL of 1x BSG. The sample was then centrifuged at 13,000 rpm (20,400 g) for 8 min. Following centrifugation, the pellet was resuspended in 2 mL 1x BSG, and the resuspended cells were loaded onto another 5 to 20% sucrose gradient. This sucrose gradient was centrifuged and the minicells were collected as described above. The sucrose gradient procedure was repeated a total of three times.

Following the final sucrose gradient step the entire minicell band was collected from the sucrose gradient and added to a 30 mL Oakridge tube that contained 10 mL of MMM buffer (200 mL 1x M9 salts, 2 mL 20% glucose, and 2.4 mL DIFCO Methionine Assay Medium). This minicell solution was centrifuged at 13,000 rpm (20,400 g) for 8 min. The pellet was resuspended in 1 mL of MMM Buffer.

The concentration of minicells was determined using a spectrophotometer. The OD_{450} was obtained by reading a sample of minicells that was diluted 1:100.

EXAMPLE 13: OTHER METHODS TO PREPARE AND ISOLATE MINICELLS

By way of non-limiting example, induction of E. coli parental cells to form minicells may occur by overexpression of the E. coli ftsZ gene. To accomplish this both plasmid-based and chromosomal overexpression constructs were created that place the ftsZ gene under the control of various regulatory elements (Table 6).

TABLE 6. REGULATORY CONSTRUCTS CONTROLLING FTSZ EXPRESSION.

Regulatory region	inducer	[inducer]	SEQ ID NO.:
Para::ftsZ	Arabinose	10 mM	1, 3
Prha::ftsZ	Rhamnose	1 mM	2, 4
Ptac::ftsZ	IPTG	30 μM	5, Garrido et al.[a]

a. Garrido, T. et al. 1993. Transcription of ftsZ oscillates during the cell cycle of Escherichia coli.

Oligonucleotide names and PCR reactions use the following format:

- "gene-1" is N-terminal, 100% homology oligo for chromosomal or cDNA amplification
- "gene-2" is C-terminal, 100% homology oligo for chromosomal or cDNA amplification
- "gene-1-RE site" is same sequence as gene-1 with additional residues for remainder of sequence, RE sites, and/or chimeric fusions.
- "gene-2-RE site" is same sequence as gene-1 with additional residues for remainder of sequence, RE sites, and/or chimeric fusions.

Use "gene-1, 2" combo for chromosomal/cDNA amplification and "gene-1 RE site, gene-2-RE site" to amplify the mature sequence from the "gene-1, 2" gel-purified product.

TABLE 7: OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 6 CONSTRUCTS

SEQ ID NO.:	Primer name	5' to 3' sequence
44	FtsZ-1	CCAATGGAACTTACCAATGACGCGG
45	FtsZ-2	GCTTGCTTACGCAGGAATGCTGGG
46	FtsZ-1-PstI	CGCGGCTGCAGATGTTTGAACCAATGGAACTTACCAATGACGCGG
47	FtsZ-2-XbaI	GCGCCTCTAGATTATTAATCAGCTTGCTTACGCAGGAATGCTGGG

Table 7 oligonucleotide sequences are for use in cloning ftsZ into SEQ ID NO.:1 and 2 (insertions of ftsZ behind the arabinose promotor (SEQ ID NO.: 1) and the rhamnose promotor (SEQ ID NO.: 2).

TABLE 8: OLIGONUCLEOTIDE PRIMER SEQUENCES FOR FTSZ CHROMOSOMAL DUPLICATION CONSTRUCTS

SEQ ID NO.:	Primer name	5' to 3' sequence
48	Kan-1	GCTAGACTGGGCGGTTTTATGGACAGCAAGC
49	Kan-2	GCGTTAATAATTCAGAAGAACTCGTCAAGAAGGCG
50	Kan-1-X-frt	GCGCCTACTGACGTAGTTCGACCGTCGGACTAGCGAAG TTCCTATACTTTCTAGAGAATAGGAACTTCGCTAGACTG GGCGGTTTTATGGACAGCAAGC
51	Kan-2-intD-frt	CAAGATGCTTTGCCTTTGTCTGAGTTGATACTGGCTTTG GGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGCGT TAATAATTCAGAAGAACTCGTCAAGAAGGCG
52	AraC-1	CGTTACCAATTATGACAACTTGACGG
53	RhaR-1	TTAATCTTTCTGCGAATTGAGATGACGCC
54	LacI^q-1	GTGAGTCGATATTGTCTTTGTTGACCAG
55	Ara-1-intD	GCCTGCATTGCGGCGCTTCAGTCTCCGCTGCATACTGTC CCGTTACCAATTATGACAACTTGACGG
56	RhaR-1-intD	GCCTGCATTGCGGCGCTTCAGTCTCCGCTGCATACTGTC CTTAATCTTTCTGCGAATTGAGATGACGCC
57	LacI^q-1-intD	GCCTGCATTGCGGCGCTTCAGTCTCCGCTGCATACTGTC CTTAATAAAGTGAGTCGATATTGTCTTTGTTGACCAG
58	FtsZ-1-X	GCCTGCATTGCGGCGCTTCAGTCTCCGCTGCATACTGTC CCGTTACCAATTATGACAACTTGACGG

In like fashion, the ftsZ gene was amplified from SEQ ID NO.: 1, 2 and Ptac::ftsZ (Garrido, T. et al. 1993. Transcription of ftsZ oscillates during the cell cycle of Escherichia coli. EMBO J. 12:3957-3965) plasmid and chromosomal constructs, respectively using the following oligonucleotides:

For amplification of araC through ftsZ of SEQ ID NO.: 1 use oligonucleotides:

AraC-1
FtsZ-2

For amplification of rhaR through ftsZ of SEQ ID NO.: 2 use oligonucleotides:

RhaR-1
FtsZ-2

For amplification of lacI^q through ftsZ of Ptac::ftsZ (Garrido, T., et al.) use oligonucleotides:

lacI^q-1
ftsZ-2

The above amplified DNA regions were gel-purified and used as template for the second round of PCR using oligonucleotides containing homology with the E. coli chromosomal gene intD and on the other end with random sequence termed “X”. Oligonucleotides used in this round of PCR are shown below:

For amplification of araC through ftsZ from SEQ ID NO.: 1 to contain homology to intD and the random X use oligonucleotides:

AraC-1-intD
FtsZ-1-X

For amplification of rhaR through ftsZ from SEQ ID NO.: 2 to contain homology to intD and the random X use oligonucleotides:

RhaR-1-intD
FtsZ-1-X

For amplification of lacIq through ftsZ from Ptac::ftsZ to contain homology to intD and the random X use oligonucleotides:

LacIq-1-intD
FtsZ-1-X

The PCR products from these PCR reactions are as shown below:

intD - araC - Ara promotor - ftsZ - “X”

intD - rhaRS - Rha promotor - ftsZ - “X”

intD - lacI^q - Ptac promotor - ftsZ - “X”

To amplify the mature complexes, the following regions were mixed and amplified with the coupled oligonucleotide sequence primers:

<u>SEQ ID NO.: 3 was produced using:</u>

intD - araC - Ara promotor - ftsZ - “X”
“X” - frt - Kan - frt - intD

AraC-1-intD
Kan-2-intD-frt

↓

intD - araC - Ara promotor - ftsZ - “X” - frt - Kan - frt - intD

SEQ ID NO.: 4 was produced using:

intD - rhaRS - Rha promotor - ftsZ - "X"
"X" - frt - Kan - frt - intD

$$\xrightarrow[\text{Kan-2-intD-frt}]{\text{RhaR-1-intD}}$$

intD - rhaRS - Ara promotor - ftsZ - "X" - frt - Kan - frt - intD

SEQ ID NO.: 5 was produced using:

intD - $lacI^q$ - Ptac promotor - ftsZ - "X"
"X" - frt - Kan - frt - intD

$$\xrightarrow[\text{Kan-2-intD-frt}]{\text{lacI}^q\text{-1-intD}}$$

intD - $lacI^q$ - Ptac promotor - ftsZ - "X" - frt - Kan - frt - intD

These expression constructs may be expressed from the plasmid, placed in single copy, replacing the native ftsZ copy on the E. coli chromosome (Garrido, T., et al. 1993. Transcription of ftsZ oscillates during the cell cycle of Escherichia coli. EMBO J. 12:3957-3965), or in duplicate copy retaining the native ftsZ copy while inserting one of the expression constructs in Table 6 into the intD gene on the same chromosome. Chromosomal duplications were constructed using the RED recombinase system (Katsenko, K. A., and B. L. Wanner. One-Step Inactivation of Chromosomal Genes in Escherichia coli K-12 Using PCR Products. Proc. Natl. Acad. Sci. 97:6640-6645. 2000) and are shown in SEQ ID NO 3-5. The later constructs allow native replication during non-minicell producing conditions, thus avoiding selective pressure during strain construction and maintenance. Furthermore, these strains provide defined points of minicell induction that improve minicell purification while creating conditions that allow strain manipulation prior to, during, and following minicell production. By way of non-limiting example these manipulations may be protein production that the cytoplasmic redox state, modify plasmid copy number, and/or produce chaperone proteins.

For minicell production, a minicell producing strain described in the previous section is grown overnight in Luria broth (LB) supplemented with 0.1% dextrose, 100 μg/ml ampicillin, and when using the single-copy ftsZ construct, 15 μM IPTG. All incubations were performed at 37°C. For minicell induction only, overnight strains are subcultured

1/1000 into the same media. If minicell induction is to be coupled with co-expression of other proteins that are controlled by a catabolite repression-sensitive regulator, dextrose was excluded. Minicell induction is sensitive to aeration and mechanical forces. Therefore, flask size, media volume and shake speed is critical for optimal yields. Likewise, bioreactor conditions must be properly regulated to optimize these production conditions.

In shake-flask cultures, strains are grown to early exponential (log) phase as monitored by optical density (OD) at 600 nm (OD_{600} 0.05-0.20). (Bioreactor conditions may differ significantly depending on the application and yield desired). For minicell induction alone, early log phase cultures are induced with the appropriate inducer concentration shown in Table 6. For coupled co-expression, these cultures are induced as shown in Table 6 for the appropriate minicell regulator, while the coupled protein(s) is induced with the inducer appropriate for the regulator controlling the synthesis of that protein. Cultures are grown under the appropriate conditions and harvested during late log (OD_{600} 0.8-1.2). Depending on the application, minicell induced cultures may be immediately chilled on ice prior to purification, or maintained at room temperature during the harvesting process.

To separate minicells from viable, parental cells, cultures are subjected to differential centrifugation (Voros, J., and R. N. Goodman. 1965. Filamentous forms of Erwinia amylovora. Phytopathol. 55:876-879). Briefly, cultures are centrifuged at 4,500 rpm in a GSA rotor for 5 min. Supernatants are removed to a fresh bottle and centrifuged at 8,000 rpm for an additional 10 min to pellet minicells. Pelleted minicells (containing contaminating parental cells) are resuspended in 2 ml LB, LBD (LB supplemented with 0.1% dextrose), Min (minimal M63 salt media) (Roozen, K. J., et al. 1971. Synthesis of ribonucleic acid and protein in plasmid-containing minicells of Escherichia coli K-12. J. Bacteriol. 107:21-23), supplemented with 0.5% casamino acids) or MDT (minimal M63 salt media, supplemented with 0.5% casamino acids, 0.1% dextrose, and thiamine). Resuspended minicells are next separated using linear density gradients. By way of non-limiting example, these gradients may contain sucrose (Cohen A., et al. 1968. The properties of DNA transferred to minicells during conjugation. Cold Spring Harb. Symp. Quant. Biol. 33:635-641), ficol, or glycerol. For example, linear sucrose gradients range from 5-20% and are poured in LB, LBD , Minor MDT. Using a SW28 swinging bucket rotor, gradients are centrifuged at 4,500 rpm for 14 min. Banded minicells are removed, mixed with LB, LBD , Minor MDT, and using a JA-20 rotor are centrifuged at 13,000 rpm for 12 min. Following centrifugation, pellets are resuspended in 2 ml LB, LBD , Minor MDT and subjected to a second density gradient. Following the second density separation, banded minicells are

removed from the gradient, pelleted as described, and resuspended in LB, LBD , Minor MDT for use and/or storage.

Purified minicells are quantitated using an OD_{600} measurement as compared to a standard curve incorporating LPS quantity, minicell size, and minicell volume. Quantitated minicells mixtures are analyzed for contaminating, viable parental cells by plating on the appropriate growth media (Table 9).

TABLE 9: MINICELL PURIFICATION AND PARENTAL CELL QUANTITATION

Purification	Total cells	Total parental cells	MC / PC ratio	Fold-purification
Before	4.76×10^{11}	3.14×10^{11}	0.25 / 1	-
After	1.49×10^{11}	6.01×10^{4}	2.48×10^{6} / 1	5.23×10^{6}

EXAMPLE 14: PROTOPLAST FORMATION

In order to allow a membrane receptor to be presented to the outside environment (displayed), minicells are made into protoplasts. In order to make the integral membrane protein receptors in the inner membrane more accessible for ligand binding, the outer membrane and cell wall were removed. The removal of the outer membrane and cell wall from E. coli whole cells and minicells to produce protoplasts was performed essentially according to previously described protocols with a few modifications (Birdsell et al., Production and Ultrastructure of Lysozyme and Ethylenediaminetetraacetate-Lysozyme Spheroplasts of Escherichia coli, J. Bacteriol. 93:427-437, 1967; Weiss et al., Protoplast Formation in Escherichia Coli, J. Bacteriol. 128:668-670, 1976. Both minicells and whole cells were processed the same way.

In brief, the cells were grown to mid-log phase and pelleted at room temperature (minicells were isolated from cultures in mid-log phase). The pellet was washed twice with 10 mM Tris. Following the second wash protoplast production may be performed using two approaches. In the first approach, following the second wash, the cells were resuspended in 100 mM Tris (pH 8.0) that contained 6-20% sucrose and put in a 37°C waterbath (the Tris/sucrose buffer was pre-warmed to 37°C). The volume used to resuspend the cells was determined by the following equation: (volume of cells x OD_{450})/ 10 = resuspension volume. After a 1 minute incubation, 2 mg/mL lysozyme was added to a final concentration of 5-100 μg/mL. The samples were then incubated for 12 minutes at 37°C while being

gently mixed. Next, 100 mM EDTA (pH 7) was slowly added over a period of 2.5 minutes (amount of EDTA added = 1/00-1/10 volume of cells) followed by a 10 min incubation at 37 °C. The protoplasts are also diluted from 20% sucrose down to either 10% or 5% sucrose, which facilitates the complete removal of the outer membrane and cell wall. The protoplasts thus generated were separated from the outer membrane and cell wall using a sucrose step gradient. A sucrose step gradient does not have a gradual increase in sucrose percentage; rather, it goes directly from one percent to the other. For example, protoplasts generated from whole cells are loaded on a step gradient that is made from 5% and 15% sucrose. The protoplasts spin through the 15% sucrose but the debris generated when making the protoplasts does not spin through the 15% sucrose. The protoplasts are thus separated from the debris. The second method to prepare protoplasts, following the second wash, 1X 10^9 cells were resuspended with 50 mM Tris, pH 8.0 containing 0.5-50 mM EDTA and 6-20% sucrose. This mixture was incubated at 37°C for10 min. Following incubation, the mixture was centrifuged at 13,200 RPM in a microcentrifuge for 2 min. After centrifugation, the pellet was resuspened in 50 mM Tris, pH 8.0 containing 5-100 μg/ml lysozyme and 6-20% sucrose. This mixture was incubated at 37°C for10 min. Following incubation, the mixture was centrifuged at 13,200 RPM in a microcentrifuge for 2 min, resuspended in 50 mM Tris pH 8.0 containing 6-20% sucrose for use.

An alternative method to remove contaminating LPS is to use affinity absorption with an anti-LPS antibody (Cortex). To accomplish this, the anti-LPS antibody was coated on either an activated agarose or sepharose matrix (Sigma) or epoxy-coated magnetic M-450 beads (Dynal). The spheroplast/protoplast mixture was subjected to the antibody coated matrix either in batch or using column chromatographic techniques to remove contaminating LPS. Following exposure, the unbound fraction(s) was collected and re-exposed to fresh matrix. To monitor the efficiency of the protoplasting reaction and LPS removal, three constructs were used (Table 10).

TABLE 10: PROTOPLAST MONITORING CONSTRUCTS

Construct	SEQ ID NO	Plasmid	SEQ ID NO	Inducible protein	Inducer
PMPX-5	6	pMPX-32	7	ΔphoA	Rhamnose
PMPX-5	6	pMPX-53	8	phoA	Rhamnose
PMPX-5	6	pMPX-33	9	toxR-phoA	Rhamnose

TABLE 11. OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 10 CONSTRUCTS

SEQ ID NO.:	Primer name	5' to 3' sequence
59	ΔphoA-1	GCCTGTTCTGGAAAACCGGGCTGCTCAGGG
60	ΔphoA-2	GCGGCTTTCATGGTGTAGAAGAGATCGG
61	ΔphoA-1-PstI	CCGCGCTGCAGATGCCTGTTCTGGAAAACCGGGCTGCT CAGGG
62	ΔphoA-2-XbaI	GCGCCTCTAGATTATTATTTCAGCCCCAGAGCGGCTTT CATGGTGTAGAAGAGATCGG
63	PhoA-1	GTCACGGCCGAGACTTATAGTCGC
64	PhoA-2	GCGGCTTTCATGGTGTAGAAGAGATCGG
65	PhoA-1-PstI	CCGCGCTGCAGATGTCACGGCCGAGACTTATAGTCGC
66	PhoA-2-XbaI	GCGCCTCTAGATTATTATTTCAGCCCCAGAGCGGCTTT CATGGTGTAGAAGAGATCGG
67	T-phoA-1-PstI	CCGCGCTGCAGATGAACTTGGGGAATCGACTGTTTATT CTGATAGCGGTCTTACTTCCCCTCGCAGTATTACTGCTC ATGCCTGTTCTGGAAAACCGGGCTGCTCAGGG
68	T-phoA-2-XbaI	GCGCCTCTAGATTATTATTTCAGCCCCAGAGCGGCTTT CATGGTGTAGAAGAGATCGG

Oligonucleotides SEQ ID NOS.:59, 60, 61 and 62 were used to amplify phoA lacking a leader sequence (ΔphoA) form the E. coli chromosome. Once amplified, this region was inserted into SEQ ID NO.: 6 using PstI and XbaI to create SEQ ID NO.: 7.

Oligonucleotides SEQ ID NOS.:63, 64, 65 and 66 were used to amplify phoA containing a leader sequence (phoA) form the E. coli chromosome. Once amplified, this region was inserted into SEQ ID NO.: 6 using PstI and XbaI to create SEQ ID NO.: 8.

Oligonucleotides SEQ ID NOS.:59, 60, 67 and 68 were used to amplify phoA lacking a leader sequence (ΔphoA) form the E. coli chromosome and form a translational fusion between the transmembrane domain of toxR from Vibrio cholerae. Once amplified, this region was inserted into SEQ ID NO.: 6 using PstI and XbaI to create SEQ ID NO.: 9.

By co-expression of minicells and protein, minicells were prepared that contained cytoplasmic PhoA (pMPX-32 expresses phoA lacking a leader sequence [ΔphoA]), periplasmic PhoA (pMPX-53 expresses native phoA that exports to the periplasmic space), or inner membrane-bound PhoA (pMPX-33 expresses phoA lacking a leader sequence fused to the transmembrane domain (TMD) of the toxR gene product from Vibrio cholerae). Using these expressed proteins, the efficiency of minicell protoplasting was monitored (Table 12).

TABLE 12. EFFICIENCY OF MINICELL PROTOPLAST PREPARATION AND PURIFICATION

Step	Location [a]	ΔPhoA	PhoA	T-PhoA	LPS total [b]
Minicell	Pellet	100	100	100	100
EDTA/lysozyme	Whole	100	100	100	100
1st Anti-LPS	Pellet	80	0	80	30
2nd Anti-LPS	Pellet	60	0	60	0

a. Measuring the location of protein being measured using an anti-BAP antibody (Sigma). Pellet refers to the presence of the expressed protein in the low-speed centrifugation pellet. These pellets contain only intact cellular bodies. Whole refers to the reaction mixture prior to low-speed centifugation.
b. Measured using a slot-blot apparatus (Bio-Rad) using the anti-LPS antibody (Cortex)

The data suggests that periplasmic PhoA is lost during the preparation, while both cytoplasmic and membrane-bound PhoA are retained in a cellular body that lacks LPS. However, during this process ~ 40% of the total minicell content is lost.

EXAMPLE 15: T7-DEPENDENT INDUCTION OF EXPRESSION

Expression from the pCAL-c expression vector is driven from a T7 bacteriophage promoter that is repressed by the LacI gene product. Transcription of the DNA into mRNA, and subsequent translation of mRNA into proteins, does not occur as long as the LacI repressor is bound to the T7 promoter. However, in the presence of IPTG, the LacI repressor does not bind the T7 promoter. Thus, induction of expression from pCAL-c sequences is dependent on the presence of IPTG. Slightly different protocols were used for the induction of Escherichia coli whole and for the induction of minicells. Slight differences are also present in the protocols for induction of minicells for ^{35}S-methionine labeling of proteins in contrast to those for the induction of minicells for Western blot analysis. These induction protocols are described bellow.

For expression in E. coli whole cells, the cells were first grown overnight in 3 mL of LB and antibiotics. The cultures were screened for the presence of the desired expression element as previously described. Cultures containing the desired expression elements were diluted 1:100 and grown to an OD_{600} of between 0.4 to 0.6. The culture size varied depending on the intended use of the cells. IPTG was then added to a final concentration of

200 μg/mL, and the cells were shaken at 30°C for 4 hours. Following the induction, cells were harvested for analysis.

The induction of minicells was carried out as follows. The minicells were diluted in MMM buffer to 1 mL total volume according to the concentration obtained from the isolation procedure (OD_{450} of about 0.5). The cells were then treated with 50 μg/mL of cycloserine for 30 minutes at 37°C to stop whole cell growth. Following the cycloserine treatment the cells were provided with an amino acid, methionine, which the MMM buffer does not contain. For ^{35}S-labeled protein induction ^{35}S-methionine was added to the minicell sample whereas, for unlabeled protein induction unlabeled methionine was added. Fifteen (15) μCi of ^{35}S-methionine (Amersham Pharmacia Biotech, Piscataway, NJ) was added to the samples for radiolabeling and 5 μmol of methionine was added to the non-labeled minicell samples. Two hundred (200) μg/mL IPTG was also added to the minicell samples, which were then shaken at 30°C for about 4 hours. Following induction, the minicells were harvested for further preparation or analysis.

EXAMPLE 16: WESTERN BLOT ANALYSIS

The CBP detection kit was purchased from Stratagene. SDS running buffer, 10% Tris-HCl ready gels, Kaleidoscope Pre-stained Standards, and Laemmli Sample Buffer were purchased from BIO RAD (Hercules, CA). GFP (FL) HRP antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Edg-3CT antibody an antibody directed to the carboxy terminus of was purchased from Exalpha Biologicals (Boston, MA). Anti-6xHis antibody, positrope, and the WesternBreeze Kit were purchased from Invitrogen (Carlsbad, CA). Protocols were carried out essentially according to the manufacturer's instructions unless otherwise indicated.

Three different Western blot protocols were used to detect protein expression in both a minicell expression system and in a whole cell expression system. For both systems, the SDS-PAGE gel and the transfer protocols were essentially as follows. The samples were denatured by diluting the samples 1:1 in Laemmli buffer (BIORAD) and then sonicated for 10 min. The denatured samples were loaded onto a 10% Tris-Glycine gel (BIORAD) and electrophoresed at 130 V for about 1.5 hours in 1X SDS running buffer (BIORAD). The electrophoresed proteins were electrotransferred to nitrocellulose membranes at 0.5 Amps for 1.5 hours in Transfer Buffer (5.8 g Tris, 2.9 g glycine, 200 mL methanol, and 3.7 mL of

10% SDS). The nitrocellulose membranes comprising the transferred proteins were used for Western bloting.

GFP Western blots were carried out as follows. The nitrocellulose membrane was blocked for 2 hours with 5% milk in PBST (PBS buffer with 0.05% Tween). Following the blocking step the nitrocellulose membrane was washed twice with PBST. For the detection of GFP protein, an anti-GFP-HRP conjugated antibody (Santa Cruz Biotechnology) was used at a dilution of 1:3000 in PBST (HRP, horse radish peroxidase). The nitrocellulose membrane was incubated in the anti-GFP-HRP antibody solution for one hour and then washed twice with PBST. GFP proteins on the nitrocellulose membrane were detected and visualized using the ECL system (Amersham).

The His-tagged Edg-1 and Edg-3 proteins were detected using a mouse anti-6xHis antibody from Invitrogen and the WesternBreeze chemoluminecent Kit (Invitrogen). The antibody was diluted 1:4000 in buffers provided by the WesternBreeze Kit. The WesternBreeze immunoblot was carried out essentially according to the manufacturer's protocol. The Edg-1-CBP and GFP-CBP fusion proteins were detected using the CBP detection Kit (Stratagene). All antibodies and substrates were provided in the Kit. Figure 3 is a photo of the Western hybridization results showing the presence of Edg-1-6xHis and Edg-3-6xHis in minicells and parent cells.

EXAMPLE 17: METHODS TO INDUCE EXPRESSION

Expression in minicells may proceed following purification of minicells and/or minicell protoplasts from parental cells and LPS constituents, repectively. However, for some applications it is suitable to co-express proteins of interest with minicell induction. For these approaches, one may use the protocol described in EXAMPLE 13 for expression of the phoA constructs. By way of non-limiting example, either of these approaches may be accomplished using one or more of the following expression constructs (Table 13).

TABLE 13: EXPRESSION CONSTRUCTS

Plasmid	Regulatory element(s)	inducer	Plasmid	SEQ ID NO.:
pMPX-5	rhaRS	Rhamnose	pUC-18	6
pMPX-7	uidR	β-glucuronate	pUC-18	10

Plasmid	Regulatory element(s)	inducer	Plasmid	SEQ ID NO.:
pMPX-8	melR	Melibiose	pUC-18	11
pMPX-18	araC	Arabinose	pUC-18	12
pMPX-6	araC	Arabinose	pUC-18	13

TABLE 14: OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 13 CONSTRUCTS

SEQ ID NO.:	Primer name	5' to 3' sequence
69	Rha-1	GCGAATTGAGATGACGCCACTGGC
70	Rha-2	CCTGCTGAATTTCATTAACGACCAG
71	Rha-1-HindIII	CGGCGAAGCTTAATTAATCTTTCTGCGAATTGAG ATGACGCCACTGGC
72	Rha-2-PstI	CGCCGTAATCGCCGCTGCAGAATGTGATCCTGCT GAATTTCATTAACGACCAG
73	Uid-1	CGCAGCGCTGTTCCTTTGCTCG
74	Uid-2	CCTCATTAAGATAATAATACTGG
75	Uid-1-HindIII	GCCGCAAGCTTCGCAGCGCTGTTCCTTTGCTCG
76	Uid-2-PstI	CCAATGCATTGGTTCTGCAGGACTCCTCATTAAG ATAATAATACTGG
77	Mel-1	CGTCTTTAGCCGGGAAACG
78	Mel-2	GCAGATCTCCTGGCTTGC
79	Mel-1-HindIII	GCCGCAAGCTTCGTCTTTAGCCGGGAAACG
80	Mel-2-SalI	CGGTCGACGCAGATCTCCTGGCTTGC
81	Ara-1	CAAGCCGTCAATTGTCTGATTCG
82	Ara-2	GGTGAATTCCTCCTGCTAGCCC
83	Ara-1-HindIII	GCGCCAAGCTTCAAGCCGTCAATTGTCTGATTCG
84	Ara-2-PstI	CTGCAGGGTGAATTCCTCCTGCTAGCCC
85	Ara-1-XhoI	GCTTAACTCGAGCTTAATAACAAGCCGTCAATTG TCTGATTC
86	Ara-2-SstI	GCTTAACCGCGGGCCAAGCTTGCATGCCTGCTCC

Oligonucleotides SEQ ID NOS.:69, 70, 71 and 72 were used to amplify the rhaRS genes and their divergent control region from the E. coli chromosome. Once amplified, this region was inserted into pUC18 using HindIII and PstI to create SEQ ID NO.: 6.

Oligonucleotides SEQ ID NOS.:73, 74, 75 and 76 were used to amplify the uidR control region, the uidR gene and the control region for expression from the E. coli chromosome. Once amplified, this region was inserted into pUC18 using HindIII and PstI to create SEQ ID NO.: 10.

Oligonucleotides SEQ ID NOS.:77, 78, 79 and 80 were used to amplify the melR gene and its divergent control region from the E. coli chromosome. Once amplified, this region was inserted into pUC18 using HindIII and SalI to create SEQ ID NO.: 11.

Oligonucleotides SEQ ID NOS.:81, 82, 83 and 84 were used to amplify the araC gene and its divergent control region from the E. coli chromosome. Once amplified, this region was inserted into pUC18 using HindIII and PstI to create SEQ ID NO.: 12.

Oligonucleotides SEQ ID NOS.:81, 82, 85 and 86 were used to amplify the araC gene and its divergent control region was PCR amplified from pBAD-24. Once amplified, this region was inserted into pEGFP (Clontech) using XhoI and SstI to create SEQ ID NO.: 13.

Except of pMPX-6, these expression constructs contain the same multiple cloning site. Therefore, any protein of interested may be inserted in each modular expression construct for simple expression screening and optimization.

By way of non-limiting example, other proteins that may be expressed are listed in Table 15.

TABLE 15: OTHER EXPRESSED PROTEINS

Protein	Origin	Construct	Purpose	SEQ ID NO.:
Edg3	Rat	native	GPCR	14
β2AR	Human	native	GPCR	15
TNFR-1a (human)	Human	residues 29-455	Receptor	18
TNFR-1b (human)	Human	residues 41-455	Receptor	17
TNF (human)	Human	native	Gene transfer	19
T-EGF	Human	chimera	Gene transfer	20
T-Invasin	Y. pseudotuberculosis	chimera	Gene transfer	21

TABLE 16: OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 15

SEQ ID NO.:	Primer name	5' to 3' sequence
87	Edg-1	GGCAACCACGCACGCGCAGGGCCACC
88	Edg-2	CAATGGTGATGGTGATGATGACCGG
89	Edg-1-SalI	CGCGGTCGACATGGCAACCACGCACGCGCAGG GCCACC
90	Edg-2-KpnI	GCGCCGGTACCTTATCAATGGTGATGGTGATG ATGACCGG
91	β2AR-1	GGGGCAACCCGGGAACGGCAGCGCC
92	β2AR-2	GCAGTGAGTCATTTGTACTACAATTCCTCC
93	β2AR-1-SalI	CGCGGTCGACATGGGGCAACCCGGGAACGGCA GCGCC
94	β2AR-2-BamHI	GCGCCGGATCCTTATTATAGCAGTGAGTCATTT GTACTACAATTCCTCC
95	TNFR(29)-1	GGACTGGTCCCTCACCTAGGGGACAGGG
96	TNFR(29)-2	CTGAGAAGACTGGGCGCGGGCGGGAGG
97	TNFR(29)-1-SalI	CGCGGGTCGACATGGGACTGGTCCCTCACCTA GGGGACAGGG
98	TNFR(29)-2-KpnI	GCGCCGGTACCTTATTACTGAGAAGACTGGGC GCGGGCGGGAGG
99	TNFR(41)-1	GATAGTGTGTGTCCCC
100	TNFR(41)-2	CTGAGAAGACTGGGCGC
101	TNFR(41)-1-NcoI	GGGAGACCATGGATAGTGTGTGTCCCC
102	TNFR(41)-2-XbaI	GCCTCATCTAGATTACTGAGAAGACTGGGCGC
103	TNF-1	GAGCACTGAAAGCATGATCCGGGACG
104	TNF-2	CAGGGCAATGATCCCAAAGTAGACCTGC
105	TNF-1-EcoRI	CCGCGGAATTCATGAGCACTGAAAGCATGATC CGGGACG
106	TNF-2-HindIII	GGCGCAAGCTTATCACAGGGCAATGATCCCAA AGTAGACCTGC
107	T-EGF-1	TCTGATAGCGGTCTTACTTCCCCTCGCAGTATT ACTGCTCAATAGTGACTCTGAATGTCCCCTGTC CCACGATGGGTACTGCCTCCATGATGGTGTGT GCATGTATATTG
108	T-EGF-2	AGGTCTCGGTACTGACATCGCTCCCCGATGTA GCCAACAACACAGTTGCATGCATACTTGTCCA ATGCTTCAATATACATGCACACACCATCATGG AGGCA
109	T-EGF-3	CCGCGGGTACCATGAACTTGGGGAATCGACTG TTTATTCTGATAGCGGTCTTACTTCCCCTCG
110	T-EGF-4	GCGCCAAGCTTATTAGCGCAGTTCCCACCACT TCAGGTCTCGGTACTGACATCGCTCCCCG
111	Inv-1	TCATTCACATTGAGCGTCACCG
112	Inv-2	TTATATTGACAGCGCACAGAGCGG
113	Inv-1-ToxR-EcoRI	GCAAGAATTCACCATGAACTTGGGGAATCGAC TGTTTATTCTGATAGCGGTCTTACTTCCCCTCG CAGTATTACTGCTCTCATTCACATTGAGCGTCA CCG

SEQ ID NO.:	Primer name	5' to 3' sequence
114	Inv-2-PstI	CGCGGTTACGTAAGCAACTGCAGTTATATTGA CAGCGCACAGAGCGG

Oligonucleotides SEQ ID NOS.:87, 88, 89 and 90 were used to amplify rat Edg3 from rat cDNA. Once amplified, this region was inserted into SEQ ID NO.: 6 (pMPX-5) using SalI and KpnI to create SEQ ID NO.:14.

Oligonucleotides SEQ ID NOS.:91, 92, 93 and 94 were used to amplify human β2 adrenergic receptor (β2AR) from human heart cDNA. Once amplified, this region was inserted into SEQ ID NO.: 6 (pMPX-5) using SalI and BamHI to create SEQ ID NO.:15.

Oligonucleotides SEQ ID NOS.:95, 96, 97 and 98 were used to amplify human tumor necrosis factor receptor (TNFR residues 29-455) from human Jurkat CL71 cDNA. Once amplified, this region was inserted into SEQ ID NO.: 12 (pMPX-18) using SalI and KpnI to create SEQ ID NO.:18.

Oligonucleotides SEQ ID NOS.:99, 100, 101 and 102 were used to amplify human tumor necrosis factor receptor (TNFR residues 41-455) from human Jurkat CL71 cDNA. Once amplified, this region was inserted into pBAD24 using NcoI and XbaI to create SEQ ID NO.:17.

Oligonucleotides SEQ ID NOS.:103, 104, 105 and 106 were used to amplify human tumor necrosis factor (TNF) from human Jurkat CL71 cDNA. Once amplified, this region was inserted into SEQ ID NO.: 13 (pMPX-6) using EcoRI and HindIII to create SEQ ID NO.:19.

TABLE 17: PROGRAM TO ANNEAL GRADIENT PCR WITH PFX POLYMERASE

Step	Temp (°C)	Time (min)
1	95	2.0
2	95	0.5
3	64	0.5
4	68	2.5
5	Goto 2, 2X	
6	95	0.5
7	62	0.5
8	68	2.5
9	Goto 6, 4X	
10	95	0.5
11	60	0.5
12	68	2.5

Step	Temp (°C)	Time (min)
13	Goto 10, 6X	
14	95	0.5
15	58	0.5
16	68	2.5
17	Goto 14, 24X	
18	4	hold
19	end	

Oligonucleotides SEQ ID NOS.:107, 108, 109 and 110 were mixed and PCR amplified using anneal gradient PCR (Table 17) to form mature human epidermal growth factor (EGF) (residues 971-1023) translationally fused to the transmembrane domain of toxR from Vibrio cholerae. Once amplified, this region was inserted into SEQ ID NO.: 13 (pMPX-6) using KpnI and HindIII to create SEQ ID NO.:20.

Using PFX polymerase (Invitrogen) oligonucleotide SEQ ID NO.:111, 112, 113 and 114 were used to amplify invasin residues 490-986 (inv) from Yersinia pseudotuberculosis chromosomal DNA and form a translational fusion between the transmembrane domain of toxR from Vibrio cholerae. Once amplified, this region was inserted into SEQ ID NO.:13 (pMPX-6) using EcoRI and PstI to create SEQ ID NO.:21.

These proteins were proof-of-principle constructs used to evaluate the minicell platform. For purposes of this initial evaluation, all proteins except TNF, T-EGF and T-Invasin were cloned into pMPX-5, with these later proteins cloned into pMPX-6 for gene transfer experiments.

Whether the approach for protein expression is co-expression with minicell induction or expression following minicell and/or protoplast isolation, the procedure to transform the expression constructs is the same. To accomplish this, protein constructs were initially cloned into E. coli MG1655 and then into the minicell producing strain of interest. Transformation events were selected prior to minicell induction. For co-induction of protein and minicells, see the protocol for phoA expression above. For post-minicell and/or protoplast purification induction experiments, following minicell purification and/or protoplast preparation and purification, these cellular bodies were induced for protein production in either LBD or MDT at a minicell or protoplast / volume ratio of 1 X 10^9 minicells or protoplasts / 1 ml media. Media was supplemented with the appropriate inducer concentration (see Table 6). Protein induction is sensitive to a variety of factors including, but not limited to aeration and temperature, thus reaction volume to surface area ratio is important, as is the method of shaking and temperature of induction. Therefore, each protein must be treated as required to optimize expression. In addition to expression parameters,

protoplasted minicells are sensitive to osmotic and mechanical forces. Therefore, protoplast protein induction reactions must also contain 10% sucrose with greater volume to surface area ratios than required for intact minicells to achieve similar aeration at lower revolutions.

Using the T-PhoA as a non-limiting example, protein expression was performed during and following minicell isolation. To accomplish this task, t-phoA co-expressed with minicell induction was compared to t-phoA expressed after minicell isolation. In both cases, overnight minicell-producing parental strains containing pMPX-5::t-phoA were subcultured into LBD supplemented with the appropriate antibiotic. Cultures were grown to OD_{600} 0.1 and induced for minicell production alone or for both minicell and protein production. Both cultures were harvested at OD_{600} 1.0 and minicells produced were harvested as described above. Minicells to be induced for T-phoA production following purification were induced by introducing 1 X 10^9 purified minicells into a 15 ml culture tube containing 1 ml MDT with 1 mM L-rhamnose. Minicell protein induction was allowed to proceed for up to 14 hours and compared to protein production obtained using the co-expression approach. For each approach, minicells were fractionated and analyzed for membrane association, total protein, and membrane association-dependent enzymatic activity. These observations were compared to post-induction, pre-isolation parental cell/minicell (PC/MC) mixtures from the co-expressed reactions. The first observation was that co-expression of minicell and protein induction was superior to post-minicell purification induction (Table 18). However, although the kinetics are slower for the post-minicell purification induction protocol, the end result is equivalent.

TABLE 18. COMPARATIVE EXPRESSION: CO-EXPRESSION VERSUS POST MINICELL PURIFICATION INDUCTION

Time of induction	Purified minicell induction [a]	Co-expression induction [a]
1.0	8.0	-
2.0	-	812.2
4.0	70.0	-
14.0	445.0	-

a. Nanogram expressed T-PhoA per 1 X 10^9 minicells.

Using the co-expression induction procedure, the amount of membrane-associated T-PhoA was measured and compared for both parental cells and minicells. Briefly, following co-expression induction of T-PhoA and minicells, minicells were purified and their membranes isolated. For membrane isolation, minicells containing expressed T-PhoA were subjected to three rounds of freeze-thaw lysis in the presence of 10 μg/ml lysozyme. Following freeze-thaw cycling, the reaction was subjected to sonication. Sonicated material was centrifuged at 6,000 rpm in a microcentrifuge for 5 min at room temperature. Supernatants were transferred to a fresh 1.5 ml Eppendorf tube and centrifuged at 70,000 rpm using a TLA-100 rotor. Following centrifugation, the pellet was resuspended in buffer and analyzed for total T-PhoA protein (Table 19) and T-PhoA enzyme activity (Table 20).

TABLE 19: MEMBRANE ASSOCIATED T-PHOA: PARENTAL CELLS VERSUS MINICELLS

Cell type [a]	Protein total [a]	T-PhoA total [b]	T-PhoA % total	Protein membrane associated [a]	T-PhoA membrane associated [b]	T-PhoA % membrane protein total
Parental cells	107.5	5.3	4.9	10.7	3.1	29.0
Minicells	4.6	0.8	17.5	1.0	0.5	50.0
Minicells EQ [b]	25.2	4.4	-	5.5	2.7	-

a. Total protein as determined by BCA assay (Pierce)

b. Microgram expressed T-PhoA per 1 X 10^9 minicells as determined via Western using an anti-PhoA antibody (Sigma) versus a PhoA standard curve (BCA determined).

c. Equivalent membrane lipid to parental cell

TABLE 20: PHOA ENZYMATIC ACTIVITY[a] (RELATIVE UNITS): PARENTAL CELLS VERSUS MINICELLS.

Cell type [b]	Unlysed	Lysed, total	Lysed, membrane
Parent cell	-	358	240
Minicell	275	265	211
Minicell EQ [c]	1,504	1,447	1,154

a. Activity determined colorimetrically using PNPP measuring optical density at 405 nm

b. Based on 1X 10^9 parental cells or minicells per reaction

c. Equivalent membrane lipid to parental cell

These results suggest that co-expression induction of T-PhoA and minicells together results in minicells containing an equivalent amount of T-PhoA produced in both parental cells and minicells. However, the percent of T-PhoA compared to total protein is 3.5X greater in minicells than in parental cells. Furthermore, of the protein made, T-PhoA constitutes 50% of the total membrane protein in minicells, whereas it is only 29% in parental cells. It should be noted that the T-PhoA protein associated with the membrane can be easily removed by treatment with mild, non-ionic detergent suggesting that the T-PhoA present in the membrane pellet is indeed associated with the membrane and not an insoluble, co-sedimenting precipitate (data not shown). Finally, PhoA is a periplasmic enzyme that requires export to the periplasmic space for proper folding and disulfide bond formation. Both of which are required for enzymatic activity. In the time course of this experiment, expression of ΔPhoA lacking a leader sequence does not demonstrate enzymatic activity. Furthermore, there is no difference between unlysed and lysed minicells containing expressed T-PhoA (Table 20) also demonstrating that the PhoA enzyme domain of the T-PhoA chimera must be present in the periplasmic space. Therefore, the T-PhoA construct must membrane associate and the PhoA domain must orient into the periplasmic space for enzymatic activity. Thus, when comparing equivalent amounts of membrane lipid between parental cells and minicells in Table 20, membrane association-dependent T-PhoA activity is almost 5X greater than in parental cells. Taking into account the data in Table 19 where 50% of T-PhoA is in the membrane compared to 29% in parental cells, the difference in T-PhoA membrane association is not sufficient to explain the almost 5X increase in minicell activity. These observations suggest that minicells contain a capacity to support more expressed membrane protein than parental cells and that the protein that associates with the membrane is more active. This activity may be simply result from minicells allowing greater efficiency of folding and disulfide bond formation for this particular protein. However, do to the fact that minicells do not contain chromosome, it is also possible that the overexpression of this protein is readily finding membrane-binding sites in the absence of chromosomally produced competitors present in parental cells. Furthermore, overexpression of proteins often leads to increased protease expression. Because minicells do not contain chromosome, these otherwise degraded surplus T-PhoA is allowed the continued opportunity to insert and

properly fold in the membrane, an attribute that could lend favor to overexpression of more complex membrane proteins.

EXAMPLE 18: EXEMPLARY METHODS TO INDUCE AND STUDY COMPLEX MEMBRANE PROTEINS

Expression of non-native (exogenous) complex membrane proteins in bacterial systems can be difficult. Using the minicell system, we are able to eliminate toxicity issues. However, issues still remain with proper translation, compartmentalization at the membrane, insertion in the membrane and proper folding for native activity. To account for these potential problems we have constructed a modular chimeric system that incorporates leader sequences and chaperone-recognized soluble domains that are native to our bacterial minicell system. In addition, we created modular constructs that overexpress the native chaperones groESL and trigger factor (tig). Finally, we have constructed minicell-producing strains that contain mutations that effect protein export and disulfide bond formation. For non-limiting examples of these constructs see Table 21.

TABLE 21: NON-LIMITING TOOLS FOR EXOGENOUS COMPLEX PROTEIN SYNTHESIS AND FUNCTION

Tool	Ref.	Residues of sequence	Purpose	SEQ ID NO
pMPX-5::phoA leader	-	1-48	Membrane targeting	22
pMPX-5::phoA leader	-	1-494	Membrane targeting	23
pMPX-5::malE leader	1	1-28	Membrane targeting	24
pMPX-5::malE leader	1	1-370	Membrane targeting	25
pMPX-17 (groESL, tig)	-	-	Chaperone	26
pMPX-5::trxA::FLAG	2	2-109 [a]	Solubility	27

a. Residues do not include FLAG sequence.

References to Table 21.

1. Grisshammer, R., et al. 1993. Expression of a rat neurotensin receptor in Escherichia coli. Biochem. J. 295:571-576.

2. Tucker, J., and R. Grisshammer. 1996. Purification of a rat neurotensin receptor expressed in Escherichia coli. Biochem. J. 317:891-899.

TABLE 22: OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 21 CONSTRUCTS

SEQ ID NO.:	Primer name	5' to 3' sequence
115	PhoA lead-1	GTCACGGCCGAGACTTATAGTCGC
116	PhoA lead-2	GGTGTCCGGGCTTTTGTCACAGG
117	PhoA lead-1-PstI	CGCGGCTGCAGATGTCACGGCCGAGACTTATAGTCGC
118	PhoA lead-2-XbaI	CGCGGTCTAGATTCTGGTGTCCGGGCTTTTGTCACAGG
119	PhoA complete	CAGCCCCAGAGCGGCTTTCATGG
120	PhoA complete-2-XbaI	CGCGGTCTAGATTTCAGCCCCAGAGCGGCTTTCATGG
121	MalE lead-1	CGCGGCTGCAGATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAACGACGATGATGTTTTCCGCCTCGGCTCTCGCCAAAATCTCTAGACGCGG
122	MalE lead-2	CCGCGTCTAGAGATTTTGGCGAGAGCCGAGGCGGAAAACATCATCGTCGTTAATGCGGATAATGCGAGGATGCGTGCACCTGTTTTTATTTTCATCTGCAGCCGCG
123	MalE-1	GGTGCACGCATCCTCGCATTATCCGC
124	MalE-2	CGGCATACCAGAAAGCGGACATCTGC
125	MalE-1-PstI	CGCGGCTGCAGATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGC
126	MalE-2-XbaI	CGCGGTCTAGAACGCACGGCATACCAGAAAGCGGACATCTGC
127	Tig-1	CGCGACAGCGCGCAATAACCGTTCTCG
128	Tig-2	GCTGGTTCATCAGCTCGTTGAAAGTGG
129	Tig-1-NarI	GCGCCGGCGCCATACGCGACAGCGCGCAATAACCGTTCTCG
130	Tig-2-XbaI	GGCGCTCTAGATTATTATTACGCCTGCTGGTTCATCAGCTCGTTGAAAGTGG
131	Gro-1	GGTAGCACAATCAGATTCGCTTATGACGG
132	Gro-2	GCCGCCCATGCCACCCATGCCGCCC
133	Gro-1-XbaI	GCGTCTAGAGGTAGCACAATCAGATTCGCTTATGACGG
134	Gro-2-HindIII	GGCGCAAGCTTATTATTACATCATGCCGCCCATGCCACCCATGCCGCCC

SEQ ID NO.:	Primer name	5' to 3' sequence
135	TrxA-1	GCGATAAAATTATTCACCTGACTGACG
136	TrxA-2	GCGTCGAGGAACTCTTTCAACTGACC
137	TrxA-1-Fxa-PstI	CGCGGCTGCAGATGATCGAAGCCCGCTCTAGA CTCGAGAGCGATAAAATTATTCACCTGACTGAC G
138	TrxA-2-FLAG-BamHI	CCGCGGGATCCTTATTAATCATCATGATCTTTA TAATCGCCATCATGATCTTTATAATCCTCGAGC GCCAGGTTAGCGTCGAGGAACTCTTTCAACTGA CC

Oligonucleotides SEQ ID NOS.:115, 116, 117 and 118 were used to amplify the phoA leader (residues 1-49) from E. coli chromosomal DNA. Once amplified, this region was inserted into SEQ ID NO.: 6 (pMPX-5) using PstI and XbaI to create SEQ ID NO.:22.

Oligonucleotides SEQ ID NOS.:115, 117, 119 and 120 were used to amplify the complete phoA gene from E. coli chromosomal DNA. Once amplified, this region was inserted into SEQ ID NO.: 6 (pMPX-5) using PstI and XbaI to create SEQ ID NO.23.

Oligonucleotides SEQ ID NOS.:121 and 122 were used to construct the malE leader (residues 1-28) sequence. Once annealed, this construct was inserted into SEQ ID NO.: 6 (pMPX-5) using PstI and XbaI to create SEQ ID NO.:24.

Oligonucleotides SEQ ID NOS.:123, 124, 125 and 126 were used to amplify the malE expanded leader (residues 1-370) from E. coli chromosomal DNA. Once amplified, this region was inserted into SEQ ID NO.: 6 (pMPX-5) using PstI and XbaI to create SEQ ID NO.:25.

Oligonucleotides SEQ ID NOS.:127, 128, 129 and 130 were used to amplify the tig control and gene region from E. coli chromosomal DNA. Once amplified, this region was ligated to the groESL amplified region below using XbaI prior to insertion into SEQ ID NO.: 6 (pMPX-5) using NarI (from the tig region) and HindIII (from the groESL region) to create SEQ ID NO.:26.

Oligonucleotides SEQ ID NOS.:131, 132, 133 and 134 were used to amplify the groESL control and gene region from E. coli chromosomal DNA. Once amplified, this region was ligated to the tig amplified region above using XbaI prior to insertion into SEQ ID NO.: 6 (pMPX-5) using NarI (from the tig region) and HindIII (from the groESL region) to create SEQ ID NO.:26.

Oligonucleotides SEQ ID NOS.:135, 136, 137 and 138 were used to amplify trxA (residues 2-109) from E. coli chromosomal DNA and insert FLAG and Factor Xa sequences. Once amplified, this region was inserted into SEQ ID NO.: 6 (pMPX-5) using PstI and BamHI to create SEQ ID NO.:27.

By way of non-limiting example, the pMPX-5::phoA leader (residues 1-48), pMPX-5::phoA leader (residues 1-494), pMPX-5::malE leader (residues 1-28), and pMPX-5::malE leader (residues 1-370) constructs are designed to direct expressed exogenous membrane proteins to the minicell cytoplasmic membrane. In addition to these constructs, By way of non-limiting example, mutations in E. coli genes secA and secY, specifically mutation prlA4 (Strader, J., et al. 1986. Kinetic analysis of lamB mutants suggests the signal sequence plays multiple roles in protein export. J. Biol. Chem. 261:15075-15080), permit promiscuous targeting to the membrane. These mutations, like the above constructs are integrated into the minicell expression system. To complement these mutations, the chaperone complex groESL and trigger factor have also been incorporated into the expression system. By way of non-limiting example, pMPX-5::trxA::FLAG will be used to create a carboxy-terminal fusion to the protein of interest to increase the membrane insertion efficiency of the membrane protein of interest (Tucker, J., and R. Grisshammer. 1996. Purification of a rat neurotensin receptor expressed in Escherichia coli. Biochem. J. 317:891-899). Also By way of non-limiting example, pMPX-5::FLAG::toxR and pMPX-5::FLAG::λcI constructs will be prepared to create a carboxy-terminal fusion to the protein of interest for use in a reporter-based assay for protein-protein interactions. By way of non-limiting example, the protein of interest for this system is a GPCR. Also By way of non-limiting example, this GPCR may be the neurotensin receptor from rat (Grisshammer, R., et al. 1993. Expression of a rat neurotensin receptor in Escherichia coli. Biochem. J. 295:571-576.), or the β2 adrenergic receptor from humans (Freissmuth, M., et al. 1991. Expression of two β-adrenergic receptors in Escherichia coli: functional interaction with two forms of the stimulatory G protein. Proc. Natl. Acad. Sci. 88:8548-8552). Insertion of a GPCR into one of these reporter constructs creates a carboxy-terminal fusion between the GPCR of interest and the DNA-binding regulatory domain of the ToxR positive activator, the λcI repressor, or the AraC positive activator. To complete this reporter system, By way of non-limiting example pMPX-5::(X)::toxR or pMPX-5::(X)::λcI will be used to create a carboxy-terminal fusion to the protein of interest for use in a reporter-based assay for protein-protein interactions, where (X) may be any protein or molecule involved in an intermolecular or intramolecular interaction. By way of non-limiting example, this molecule of interest may be a G-protein.

This G-protein may be the $G\alpha_{i1}$-protein from rat (Grisshammer, R., and E. Hermans. 2001. Functional coupling with Gαq and Gαi1 protein subunits promotes high-affinity agonist binding to the neurotensin receptor NTS-1 expressed in Escherichia coli. FEBS Lett. 493:101-105), or the $G_{s\alpha}$-protein from human (Freissmuth, M., et al. 1991. Expression of two β-adrenergic receptors in Escherichia coli: functional interaction with two forms of the stimulatory G protein. Proc. Natl. Acad. Sci. 88:8548-8552). Like the GPCR, insertion of a G-protein into one of these reporter constructs creates a carboxy-terminal fusion between the G-protein of interest and the DNA-binding regulatory domain of the ToxR positive activator, the λcI repressor, or other regulatory protein. Finally, these plasmid constructs contain the DNA-binding domain of each regulator; the ctx regulatory region from Vibrio cholerae (Russ, W. P., and D. M. Engelman. 1999. TOXCAT: a measure of transmembrane helix association in a biological membrane. 96:863-868), or the P_R1O_R1 region of bacteriophage lambda (Hu, J. C., et al. 1990. Sequence requirements for coiled-coils: analysis with lambda repressor-GCN4 leucine zipper fusions. Science. 250:1400-1403), respectively. By way of non-limiting example, each binding domain is coupled to a reporter sequence encoding luciferase (Dunlap, P. V., and E. P. Greenberg. 1988. Control of Vibrio fischeri lux gene transcription by a cyclic AMP receptor protein-luxR protein regulatory circuit. J. Bacteriol. 170:4040-4046), green fluorescent protein (GFP) (Yang, T. T., et al. 1996. Dual color microscopic imagery of cells expressing the green fluorescent protein and a red-shifted variant. Gene. 173:19-23; Matthysse, A. G., et al. 1996. Construction of GFP vectors for use in gram-negative bacteria other than Escherichia coli. FEMS Microbiol. Lett. 145:87-94), or other reporter. Co-expression of these GPCR and G-protein chimeras will create a system measuring the interaction between a GPCR and G-protein within an intact minicell. This system is designed to be used as a positive or negative read-out assay and may be used to detect loss or gain of GPCR function. Although the GPCR-G-protein interaction is provided as an example, this modular system may be employed with any soluble or membrane protein system measuring protein-protein or other intermolecular interaction.

EXAMPLE 19: EXEMPLARY METHODS FOR GENE TRANSFER USING MINICELLS OR MINICELL PROTOPLASTS

Included in the design of the invention is the use of minicells to transfer genetic information to a recipient cell. By way of non-limiting example, this gene transfer may occur between a minicell and a mammalian cell in vitro, or in vivo, and this gene transfer may

occur through cell-specific interactions, through general interactions, or a combination of each. To accomplish this task three basic constructs were created. Each of these constructs is created in pMPX-6 which contains a CMV promotor controlling the synthesis of GFP. The plasmid pMPX-6 was constructed by cloning the araC through the multiple cloning site of pBAD24 into pEGFP (Clontech). This construct provided a bacterial regulator as well as a method to monitor the success of gene transfer using GFP expression form the CMV promotor. In design, the protein expressed using the bacterial promotor will drive the cell-cell interaction, while the successful transfer of DNA from the minicell to the recipient cell will initiate the production of GFP. By way of non-limiting example, proteins that will drive the cell-cell interaction may be the invasin protein from Yersinia pseudotuberculosis, which stimulates β1 integrin-dependent endocytic events. To properly display the invasin protein on the surface of minicells, the domain of invasin that stimulates these events (residues 490-986) (Dersch, P., and R. R. Isberg. 1999. A region of the Yersinia pseudotuberculosis invasin protein enhances integrin-mediated uptake into mammalian cells and promotes self-association. EMBO J. 18:1199-1213) was fused to the transmembrane domain of ToxR. Expression of this construct from pMPX-6 will display T-Inv on the surface of the minicell and stimulate endocytosis with any cell displaying a β1 integrin. Thus, T-Inv display will provide a general mechanism of gene transfer from minicells. To provide specificity, By way of non-limiting example, the ligand portion of epidermal growth factor (EGF) may be fused to the transmembrane domain of ToxR, thus creating a protein that will interact with cells displaying the EGF receptor (EGFR). Likewise, tumor nucrosis factor (TNF) may also serve this purpose by stimulating cell-cell interactions between minicells displaying TNF and cells displaying TNF receptor (TNFR). Although EGF-EGFR and TNF-TNFR interactions may stimulate cell-cell fusion between minicells and recipient cells, or minicell uptake, this alone may not be sufficient to efficiently transfer genetic information from minicells. Therefore, a genetic approach to increasing the cell-cell genetic transfer may be the development of a genetic switch that senses the specificity interaction, e.g. EGF-EGFR interaction, and turns on the production of a second gene product, e.g. invasin, that stimulates the endocytic event. By way of non-limiting example, this genetic switch may be similar to the GPCR-G-protein interaction reporter system above, in that an extracellular event stimulates the dimerization of a transcriptional active regulator, thus turning on the production of invasin or invasin-like protein. In either approach, the display system to stimulate transfer of genetic information from minicells to recipient cells may also be applicable to the transfer of substances other than genetic information, e.g. pre-synthesized therapeutic drugs.

To test this targeting methodology, different pMPX-6 constructs containing each of these general or specific cell-cell interaction proteins will be transformed into a minicell producing strain and either by co-expression induction of minicells, by post-minicell purification induction, or by post-protoplasting induction, minicells displaying the targeting protein of interest will be produced. When using the co-expression induction and post-minicell purification induction of the targeting protein approaches, it is necessary to protoplast the purified minicells after protein induction. Once the targeting protein has been displayed on the surface of a minicell protoplast, these protoplasts are ready to be exposed to target cells. For preliminary experiments these interactions will be monitored using cell culture of Cos cells in comparison to lipofectamine (Invitrogen), electroporation, and other transfection techniques. Initial experiments will expose protoplasts displaying T-Inv to Cos cells and compare the transfection efficiency to protoplast containing pMPX-6::t-inv in the absence of t-inv expression, naked pMPX-6::t-inv alone, and naked pMPX-6::t-inv with lipofectamine. Each of these events will be monitored using fluorescent microscopy and/or flow cytometry. From these results the specific targeting apparatus proteins will be tested. Using A-431 (display EGFR) and K-562 (no EGFR) cell lines, the pMPX-6::t-egf constructs will be tested. Using the same approaches as for the t-inv study, the level of transfection between A-431 and K-562 cell lines will be measured and compared to those achieved using lipofectamine. Similarly, the ability of TNF to stimulate gene transfer will be studied using L-929 cells. In all cases, the ability of these general and specific targeting protein constructs will be compared to standard transfection techniques. Upon positive results, these methodologies will be tested on difficult to transfect cell lines, e.g. adult cardiomyocytes. The basis of these results will create a foundation for which applications into in vivo gene transfer may occur.

EXAMPLE 20: ADDITIONAL AND OPTIMIZED METHODS FOR GENETIC EXPRESSION

Expression in minicells may occur following purification of minicells and/or minicell protoplasts from parental cells and LPS constituents, respectively. However, for some applications it is preferred to co-express proteins of interest with minicell induction. For these approaches, one may use the protocol described in Example 13 for expression of the phoA constructs. Either of these approaches may be accomplished using one or more of the following expression constructs (Table 23) and/or optimized expression constructs (Table 25).

Expression plasmid pCGV1 contains a temperature sensitive lambda cI repressor (cI857) and both lambda PR and PL promoters (Guzman, C. A., et al. 1994. A novel Escherichia coli expression-export vector containing alkaline phosphatase as an insertional inactivation screening system. Gene. 148:171-172) with an atpE initiation region (Schauder, B., et al. 1987. Inducible expression vectors incorporating the Escherichia coli atpE translational initiation region. Gene. 52:279-283). Included in the design of the invention is the modification of this expression vector to best align the required Shine-Delgarno ribosomal binding site with cloning sites. In addition, the pCGVI expression vector was modified to incorporate a stem-loop structure at the 3-prime end of the transcript in order to provide a strong transcriptional stop sequence (Table 23).

Expression plasmid pCL478 contains a temperature sensitive lambda cI repressor (cI857) and both lambda PR and PL promoters (Love, C. A., et al. 1996. Stable high-copy bacteriophage promoter vectors for overproduction of proteins in Escherichia coli. Gene. 176:49-53). Included in the design of the invention is the modification of this expression vector to best align the required Shine-Delgarno ribosomal binding site with cloning sites. In addition, the pCL478 expression vector was modified to incorporate a stem-loop structure at the 3-prime end of the transcript in order to provide a strong transcriptional stop sequence (Table 23).

TABLE 23. LAMBDA CI857 EXPRESSION VECTOR MODIFICATIONS

New Plasmid	Parent plasmid	Region removed	Region added [a]	SEQ ID NO
pMPX-84	pCGV1	NdeI - BamHI	NdeI, SD - PstI, XbaI, KpnI, Stem-loop, BamHI	139
pMPX-85	pCGV1	NdeI - BamHI	NdeI, SD - SalI, XbaI, KpnI, Stem-loop, BamHI	140
pMPX-86	pCL478	BamHI - XhoI	BamHI, SD - PstI, XbaI, KpnI, Stem-loop, XhoI	141
pMPX-87	pCL478	BamHI - XhoI	BamHI, SD - SalI, XbaI, KpnI, Stem-loop, XhoI	142

a. "SD" refers to a Shine-Delgarno ribosome-binding sequence; "Stem-loop" refers to a stem-loop structure that functions as a transcriptional stop site.

TABLE 24. OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 23

SEQ ID NO	Primer name	5' to 3' sequence
143	CGV1-1-SalI	TATGTAAGGAGGTTGTCGACCGGCTCAGTCTAGAGGTACCCGCCCTCA TCCGAAAGGGCGTATTG
144	CGV1-2-SalI	GATCCAATACGCCCTTTCGGATGAGGGCGGGTACCTCTAGACTGAGCC GGTCGACAACCTCCTTACA
145	CGV1-1-PstI	TATGTAAGGAGGTTCTGCAGCGGCTCAGTCTAGAGGTACCCGCCCTCA TCCGAAAGGGCGTATTG
146	CGV1-2-PstI	GATCCAATACGCCCTTTCGGATGAGGGCGGGTACCTCTAGACTGAGCC GCTGCAGAACCTCCTTACA
147	CL478-1-SalI	GATCCTAAGGAGGTTGTCGACCGGCTCAGTCTAGAGGTACCCGCCCTC ATCCGAAAGGGCGTATTC
148	CL478-2-SalI	TCGAGAATACGCCCTTTCGGATGAGGGCGGGTACCTCTAGACTGAGCC GGTCGACAACCTCCTTAG
149	CL478-1-PstI	GATCCTAAGGAGGTTCTGCAGCGGCTCAGTCTAGAGGTACCCGCCCTC ATCCGAAAGGGCGTATTC
150	CL478-2-PstI	TCGAGAATACGCCCTTTCGGATGAGGGCGGGTACCTCTAGACTGAGCC GCTGCAGAACCTCCTTAG

Oligonucleoides SEQ ID NOS.: 143 and 144 were annealed to each other to generate a DNA molecule with a 5' overhang at both ends. The overhangs are designed so that the DNA can be directly cloned into pCGVI cut with NdeI (5' overhang is TA) and BamHI (5' overhang is GATC). Insertion of the annealed DNA into pCGVI creates SEQ ID NO.: 139, pMPX-84.

Oligonucleoides SEQ ID NOS.: 145 and 146 were annealed to each other to generate a DNA molecule with a 5' overhang at both ends. The overhangs are designed so that the DNA can be directly cloned into pCGVI cut with NdeI (5' overhang is TA) and BamHI (5' overhang is GATC). Insertion of the annealed DNA into pCGVI creates SEQ ID NO.: 140, pMPX-85.

Oligonucleoides SEQ ID NOS.: 147 and 148 were annealed to each other to generate a DNA molecule with a 5' overhang at both ends. The overhangs are designed so that the DNA can be directly cloned into pCL478 cut with BamHI (5' overlap is GATC) and XhoI (overhang is TCGA). Insertion of the annealed DNA into pCL578 cut with BamHI and XhoI creates SEQ ID NO.: 141, pMPX-86.

Oligonucleoides SEQ ID NOS.: 149 and 150 were annealed to were annealed to each other to generate a DNA molecule with a 5' overhang at both ends. The overhangs are designed so that the DNA can be directly cloned into pCL578 cut with BamHI (5' overlap is

GATC) and XhoI (overhang is TCGA). Insertion of the annealed DNA into pCL478 cut with BamHI and XhoI creates SEQ ID NO.: 142, pMPX-87.

The optimized expression constructs in Table 25 were created from SEQ ID NOS.: 6, 11, and 12 (see Table 13). Modifications were made to optimize the alignment of the SalI or PstI cloning sites with the Shine-Delgarno ribosome-binding site. In addition, stem-loop transcriptional termination sequences were added on the 3' end of the cloning region.

TABLE 25: EXPRESSION CONSTRUCTS

Plasmid	Regulatory element(s)	inducer	Plasmid	SEQ ID NO.:
pMPX-67	RhaRS	Rhamnose	PUC-18	151
pMPX-72	RhaRS	Rhamnose	PUC-18	152
pMPX-66	AraC	Arabinose	PUC-18	153
pMPX-71	AraC	Arabinose	PUC-18	154
pMPX-68	MelR	Melibiose	PUC-18	155

TABLE 26. OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 25 CONSTRUCTS

SEQ ID NO.:	Primer name	5' to 3' sequence
69	Rha-1	GCGAATTGAGATGACGCCACTGGC
156	Rha-SD	GCAGAACCTCCTGAATTTCATTACGACC
71	Rha-1-HindIII	CGGCGAAGCTTAATTAATCTTTCTGCGAATTGAG ATGACGCCACTGGC
157	Rha-SD SalI KpnI	CCGCGGGTACCAATACGCCCTTTCGGATGAGGGC GCGGGGATCCTCTAGAGTCGACGTCGACAACCTC CTGAATTTCATTACGACC
158	Rha-SD KpnI KpnI	CCGCGGGTACCAATACGCCCTTTCGGATGAGGGC GCGGGGATCCTCTAGAGTCGACCTGCAGAACCTC CTGAATTTCATTACGACC
81	Ara-1	CAAGCCGTCAATTGTCTGATTCG
159	Ara-SD	CTGCAGGGCCTCCTGCTAGCCCAAAAAAACGGG TATGG
83	Ara-1-HindIII	GCGCCAAGCTTCAAGCCGTCAATTGTCTGATTCG
160	Ara-SD SalI KpnI	CCGCGGGTACCAATACGCCCTTTCGGATGAGGGC GCGGGGATCCTCTAGAGTCGACGTCGACGGCCTC CTGCTAGCCCAAAAAAACGGGTATGG

SEQ ID NO.:	Primer name	5' to 3' sequence
161	Ara-SD PstI KpnI	CCGCGGGTACCAATACGCCCTTTCGGATGAGGGC GCGGGGATCCTCTAGAGTCGACCTGCAGGGCCTC CTGCTAGCCCAAAAAAACGGGTATGG
77	Mel-1	CGTCTTTAGCCGGGAAACG
162	Mel-SD	CCTCCTGGCTTGCTTGAATAACTTCATCATGG
79	Mel-1-HindIII	GCCGCAAGCTTCGTCTTTAGCCGGGAAACG
163	Mel-SD-SalI KpnI	CCGCGGGTACCAATACGCCCTTTCGGATGAGGGC GCGGGGATCCTCTAGAGTCGACCCCCTCCTGGCT TGCTTGAATAACTTCATCATGGC

Oligonucleotides SEQ ID NOS.: 69, 156, 72, and 157 were used to amplify the rhaRS genes and their divergent control region from the E. coli chromosome and insertion of an optimized SalI-Shine-Delgarno ribosome-binding alignment and a stem-loop transcriptional termination sequence. Once amplified, this region was inserted into pUC18 using HindIII and KpnI to create pMPX67, SEQ ID NO.: 151.

Oligonucleotides SEQ ID NOS.: 69, 156, 72, and 158 were used to amplify the rhaRS genes and their divergent control region from the E. coli chromosome and insertion of an optimized PstI-Shine-Delgarno ribosome-binding alignment and a stem-loop transcriptional termination sequence. Once amplified, this region was inserted into pUC18 using HindIII and KpnI to create, pMPX-72, SEQ ID NO.: 152.

Oligonucleotides SEQ ID NOS.: 81, 159, 81, 160 were used to amplify the araC genes and their divergent control region from the E. coli chromosome and insertion of an optimized SalI-Shine-Delgarno ribosome-binding alignment and a stem-loop transcriptional termination sequence. Once amplified, this region was inserted into pUC18 using HindIII and KpnI to create, pMPX-66, SEQ ID NO.: 153.

Oligonucleotides SEQ ID NOS.: 81, 159, 81, 161 were used to amplify the araC genes and their divergent control region from the E. coli chromosome and insertion of an optimized PstI-Shine-Delgarno ribosome-binding alignment and a stem-loop transcriptional termination sequence. Once amplified, this region was inserted into pUC18 using HindIII and KpnI to createm pMPX-71, SEQ ID NO.: 154.

Oligonucleotides SEQ ID NOS.: 77, 162, 79, 163 were used to amplify the melR genes and their divergent control region from the E. coli chromosome and insertion of an optimized SalI-Shine-Delgarno ribosome-binding alignment and a stem-loop transcriptional termination sequence. Once amplified, this region was inserted into pUC18 using HindIII and KpnI to create, pMPX-68, SEQ ID NO.: 155.

EXAMPLE 21: OPTIMIZATION OF RAT NEUROTENSIN RECEPTOR (NTR) EXPRESSION

Expression of specific GPCR proteins in minicells may require chimeric domain fusions to stabilize the expressed protein and/or direct the synthesized protein to the membrane. The NTR protein from rat was cloned into several chimeric combinations to assist in NTR expression and membrane association (Grisshammer, R., et al. 1993. Expression of a rat neurotensin receptor in Escherichia coli. Biochem. J. 295:571-576; Tucker, J., and Grisshammer, R. 1996. Purification of a rat neurotensin receptor expressed in Escherichia coli. Biochem. J. 317:891-899). Methods for construction are shown the Tables below.

TABLE 27. NEUROTENSIN RECEPTOR EXPRESSION FACILITATING CONSTRUCTS

Protein [a]	Construct [b]	SEQ ID NO
MalE(L)	SalI-MalE (1-370)-Factor Xa-NTR homology	164
NTR	Factor Xa-NTR (43-424)-NotI-FLAG-KpnI	165
MalE(L)-NTR	SalI-MalE(1-370)-Factor Xa-NTR(43-424)-NotI-FLAG-KpnI	166
MalE(S)-NTR	SalI-MalE(1-28)-Factor Xa-NTR(43-424)-NotI-FLAG-KpnI	167
TrxA	NotI-TrxA(2-109)-NotI	168
MalE(L)-NTR-TrxA	SalI-MalE(1-370)-Factor Xa-NTR(43-424)-NotI-TrxA(2-109)-FLAG-KpnI	169
MalE(S)-NTR-TrxA	SalI-MalE(1-28)-Factor Xa-NTR(43-424)-NotI-TrxA(2-109)-FLAG-KpnI	170

a. (L) refers to MalE residues 1-370, and (S) refers to MalE residues 1-28.
b. All mature constructs were cloned into SalI and KpnI sites of SEQ ID NOS.: 140, 142, 151 and 153.

TABLE 28. OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 27

SEQ ID NO.:	Primer name	5' to 3' sequence
171	MalE-1	GGTGCACGCATCCTCGCATTATCCGC
172	MalE-2	CGCACGGCATACCAGAAAGCGGACATCTGCG
173	MalE-1-SalI	CCGCGGTCGACATGAAAATAAAAACAGGTGCACGC ATCCTCGC
174	MalE-2-XaNTR	GCCGTGTCGGATTCCGAGGTGCGGCCTTCGATACGC ACGGCAT ACCAAGAAAGCGGGATGTTCGGC
175	NTR-1	CCTCGGAATCCGACACGGCAGGGC
176	NTR-2	GTACAGGGTCTCCCGGGTGGCGCTGG
177	NTR-1-Xa	CCGCGATCGAAGGCCGCACCTCGGAATCCGACACG GCAGGGCC
178	NTR-2-Flag	GGCGCGGTACCTTTGTCATCGTCATCTTTATAATCT GCGGCCGC GTACAGGGTCTCCCGGGTGGCGCTGGTGG
179	NTR-2-Stop KpnI	GCGGCGGTACCTTATTATTTGTCATCGTCATCTTTAT AATCTGC GGCCGCG
180	NTR-1-Xa Lead	CCGCATTAACGACGATGATGTTTTCCGCCTCGGCTC TCGCCAAA ATCATCGAAGGCCGCACCTCGGAATCCGACACGGC
181	NTR-2-Lead2 SalI	CCGCGGTCGACATGAAAATAAAAACAGGTGCACGC ATCCTCGC ATTATCCGCATTAACGACGATGATGTTTTCCGCCTC GGC
182	TrxA-1	CCGCGAGCGATAAAATTATTCACCTGACTGACG
183	TrxA-2	GCCCGCCAGGTTAGCGTCGAGGAACTCTTCAACTG ACC
184	TrxA-1-NotI	GCGGCCGCAAGCGATAAAATTATTCACCTGACTGA CG
185	TrxA-2-NotI	GGCGCTGCGGCCGCATCATCATGATCTTTATAATCG CC

Oligonucleotides SEQ ID NOS.: 171, 172, 173 and 174 were used to amplify malE residues 1-370 from the E. coli chromosome to create SEQ ID NO.: 164. Using overlap PCR with the extended NTR homology, a chimeric translational fusion was made between MalE (1-370) and NTR residues 43-424 (SEQ ID NO.: 165) to create a SEQ ID NO.: 166. SEQ ID NO.: 166 was cloned into plasmids pMPX-85, pMPX-87, pMPX-66 and pMPX-67 (respectively, SEQ ID NOS.: 140, 142, 151 and 153) using SalI and KpnI.

Three-step PCR with oligonucleotides, SEQ ID NOS.: 175 and 176 as primers was used to amplify NTR residues 43-424 from rat brain cDNA. SEQ ID NOS.: 177 and 178 were then used with the NTR (43-424) template to add factor Xa and FLAG sequence.

Finally, SEQ ID NOS.: 177 and 179 were used to add a KpnI site to create SEQ ID NO.: 165. Using overlap PCR with malE(1-370) containing extended NTR homology, a chimeric translational fusion was made between NTR (43-424) and MalE (1-370) (SEQ ID NO.: 164) to create a SEQ ID NO.: 166. SEQ ID NO.: 166 was cloned into SEQ ID NOS.: 140, 142, 151 and 153 using SalI and KpnI.

Using three-step PCR oligonucleotides SEQ ID NOS.: 175 and 176 were first used to amplify NTR residues 43-424 from rat brain cDNA. SEQ ID NOS.: 178 and 180 were then used with the NTR (43-424) template to add factor Xa and FLAG sequence. Finally, SEQ ID NOS.: 179 and 181 were used to add KpnI to create SEQ ID NO.: 167. SEQ ID NO.: 167 was cloned into SEQ ID NOS.: 140, 142, 151 and 153 using SalI and KpnI.

Oligonucleotides SEQ ID NOS.: 182, 183, 184 and 185 were used to amplify TrxA residues 2-109 from the E. coli chromosome to create SEQ ID NO.: 168. Using NotI, TrxA residues 2-109 was cloned into SEQ ID NOS.: 166 and 167 to create SEQ ID NOS.: 169 and 170, respectively. SEQ ID NO.: 169 and 170 were cloned into SEQ ID NOS.: 140, 142, 151 and 153 using SalI and KpnI.

EXAMPLE 22: METHODS FOR FUNCTIONAL GPCR ASSAY

Functional G-protein-coupled receptor (GPCR) binding assays in minicells requires expression of a GPCR of interest into the minicell membrane bilayer and cytoplasmic expression of the required G-protein. For these purposes, constructs were created to co-express both a GPCR and a G-protein. To regulate the ratio of GPCR to G-protein, transcriptional fusions were created. In these constructs, the GPCR and G-protein are co-transcribed as a bi-cistronic mRNA. To measure the GPCR-G-protein interaction in the intact minicell, each protein was created as a chimera with a transactivation domain. For these studies the N-terminal DNA-binding, activation domain of the ToxR protein from V. cholerae was fused to the C-terminus of both the GPCR and G-protein. Finally, to measure the interaction GPCR-G-protein interaction, the ToxR-activated ctx promoter region was cloned in front of lacZ. Dimerization of the ToxR DNA-binding region will bind and activate the ctx promoter. In this construct, heterodimerization of the GPCR and G-protein will promote dimerization of ToxR that will be monitored by LacZ expression. Details of these constructs are shown in Table 29.

TABLE 29. FUNCTIONAL HUMAN GPCR CONSTRUCTS

Protein [a, b]	Construct [a, b]	SEQ ID NO.:
β2AR	SalI-β2AR-PstI, XhoI	186
GS1α	XhoI-GS1α–XbaI	187
β2AR-GS1α fusion	SalI-β2AR-PstI, XhoI-GS1α–XbaI	188
β2AR-stop	SalI-β2AR-PstI-Stop-SD-XhoI	189
β2AR-stop-GS1α	SalI-β2AR-PstI-Stop-SD-XhoI-GS1α–XbaI	190
ToxR	ClaI-ToxR-XbaI	191
GS1α	XhoI-GS1α-ClaI	192
GS2α	XhoI-GS2α-ClaI	193
Gαq	XhoI-Gqα-ClaI	194
Giα	XhoI-Giα-ClaI	195
Gα12/13	XhoI-Gα12/13-ClaI	196
GS1α-ToxR	XhoI-GS1α-ClaI-ToxR-XbaI	197
GS2α-ToxR	XhoI-GS2α-ClaI-ToxR-XbaI	198
Gαq-ToxR	XhoI- Gαq -ClaI-ToxR-XbaI	199
Giα-ToxR	XhoI-Giα-ClaI-ToxR-XbaI	200
Gα12/13-ToxR	XhoI- Gα12/13-ClaI-ToxR-XbaI	201
ToxR	PstI-ToxR-XhoI	202
β2AR	SalI-β2AR-PstI	203
β2AR-ToxR	SalI-β2AR-PstI-ToxR-Stop-SD-XhoI	204
β2AR-ToxR-stop-GS1α-ToxR	SalI-β2AR-PstI-ToxR-Stop-SD-XhoI-GS1α-ClaI-ToxR-XbaI	205
Pctx	XbaI-Pctx-lacZ homology	206
lacZ	Pctx homology-lacZ-XbaI	207
Pctx::lacZ	XbaI-Pctx-lacZ-XbaI	208

a. "SD" refers to the Shine-Delgarno ribosome-binding sequence and "ToxR" refers to the transactivation, DNA-binding domain of the ToxR protein (residues 5-141).

b. All mature constructs were cloned into SalI and XbaI sites of SEQ ID NOS.: 140, 142, 151 and 153.

TABLE 30. OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 29.

SEQ ID NO.:	Primer name	5' to 3' sequence
209	β2AR-1	GGGGCAACCCGGGAACGGCAGCGCC
210	β2AR-2	GCAGTGAGTCATTTGTACTACAATTCCTCC
211	β2AR-1-SalI	CGCGGTCGACATGGGGCAACCCGGGAACGGCAGCGCC
212	β2AR-2-Link-XhoI	GGCTCGAGCTGCAGGTTGGTGACCGTCTGGCCACGCTC TAGCAGTGAGTCATTTGTACTACAATTCC
213	GS1α-1	GGGCTGCCTCGGGAACAGTAAGACCGAGG
214	GS1α-2	GAGCAGCTCGTACTGACGAAGGTGCATGC
215	GS1α-1-XhoI	GGAGGCCCTCGAGATGGGCTGCCTCGGGAACAGTAAG ACCGAGG

SEQ ID NO.:	Primer name	5' to 3' sequence
216	GS1α-2-XbaI	CCTCTAGATTATTATCGATGAGCAGCTCGTACTGACGA AGGTGCATGC
217	GS1α-2-ClaI	CCATCGATGAGCAGCTCGTACTGACGAAGGTGCATGC
218	Gα12-1	CCGGGGTGGTGCGGACCCTCAGCCGC
219	Gα12-2	CTGCAGCATGATGTCCTTCAGGTTCTCC
220	Gα12-1-XhoI	GCGGGCTCGAGATGTCCGGGGTGGTGCGGACCCTCAGC CGC
221	Gα12-2-ClaI	GCGCCATCGATCTGCAGCATGATGTCCTTCAGGTTCTCC
222	Gαq-1	GACTCTGGAGTCCATCATGGCGTGCTGC
223	Gαq-2	CCAGATTGTACTCCTTCAGGTTCAACTGG
224	Gαq-1-XhoI	ATGACTCTGGAGTCCATCATGGCGTGCTGC
225	Gαq-2-ClaI	GCGCCATCGATGACCAGATTGTACTCCTTCAGGTTCAACT GG
226	Giα-1	GGGCTGCACCGTGAGCGCCGAGGACAAGG
227	Giα-2	CCTTCAGGTTGTTCTTGATGATGACATCGG
228	Giα-1-XhoI	ATGGGCTGCACCGTGAGCGCCGAGGACAAGG
229	Giα-2-ClaI	GCGCCATCGATGAAGAGGCCGCAGTCCTTCAGGTTGTTCT TGA TGATGACATCGG
230	GS2α-1	GGGCTGCCTCGGGAACAGTAAGACCGAGG
231	GS2α-2	GAGCAGCTCGTACTGACGAAGGTGCATGC
232	GS2α-1-XhoI	ATGGGCTGCCTCGGGAACAGTAAGACCGAGG
233	GS2α-2-ClaI	GCGCCATCGATGAGCAGCTCGTACTGACGAAGGTGCATG C
234	β2AR-2-Link-Stop-XhoI	GGCTCGAGGGCCTCCTTGATTATTACTCGAGGGCCTCC TTGATTATTACTGCAGGTTGGTGACCGTCTGGCCACGC TCTAGCAGTGAGTCATTTGTACTACAATTCC
235	β2AR-2-Link	CCCTGCAGGTTGGTGACCGTCTGGCCACGCTCTAGCAG TGAGTCATTTGTACTACAATTCC
236	Tox (5-141)-1B	GGACACAACTCAAAAGAGATATCGATGAGTCATATTG G
237	Tox (5-141)-2	GAGATGTCATGAGCAGCTTCGTTTTCGCG
238	Tox (5-141)-1-Link	GCGTGGCCAGACGGTCACCAACCTGCAGGGACACAAC TCAAAAGAGATATCG
239	Tox (5-141)-2-XhoI	CGGGGATCCTCTAGATTATTAAGAGATGTCATGAGCAG CTTCGTTTTCGCG
240	Ctx-1	GGCTGTGGGTAGAAGTGAAACGGGGTTTACCG
241	Ctx-2	CTTTACCATATAATGCTCCCTTTGTTTAACAG
242	Ctx-2-XbaI	CGCGGTCTAGAGGCTGTGGGTAGAAGTGAAACGGGGT TTACCG
243	Ctx-2-LacZ	CGACGGCCAGTGAATCCGTAATCATGGTCTTTACCATA TAATGCTCCCTTTGTTTAACAG
244	LacZ-1	CCATGATTACGGATTCACTGGCCGTCG

SEQ ID NO.:	Primer name	5' to 3' sequence
245	LacZ-2	CCAGACCAACTGGTAATGGTAGCGACC
246	LacZ-1-Ctx	GGTAAAGACCATGATTACGGATTCACTGGCCGTCG
247	LacZ-2-XbaI	GCGCCTCTAGAAATACGCCCTTTCGGATGAGGGCGTT ATTATTTTTGACACCAGACCAACTGGTAATGGTAGCG ACC

Oligonucleotides SEQ ID NOS.: 209, 210, 211 and 212 were used to amplify human β2AR from human cDNA to create SEQ ID NO.: 186. Using SalI and XhoI a translational fusion was made between β2AR and human GS1α (SEQ ID NO.: 187) to create a SEQ ID NO.: 188. SEQ ID NO.: 188 was cloned into SEQ ID NOS.: 140, 142, 151 and 153 using SalI and XbaI.

Oligonucleotides SEQ ID NOS.: 213, 214, 215 and 216 were used to amplify human GS1α from human cDNA to create SEQ ID NO.: 187. Using XhoI and XbaI a translational fusion was made between GS1α and human β2AR (SEQ ID NO.: 186) create SEQ ID NO.: 188. SEQ ID NO.: 188 was cloned into SEQ ID NOS.: 140, 142, 151and 153 using SalI and XbaI.

Oligonucleotides SEQ ID NOS.: 213, 214, 215 and 217 were used to amplify human GS1α from human cDNA to create SEQ ID NO.: 192. Using XhoI and XbaI a translational fusion was made with ToxR residues 5-141 from Vibrio cholerae (SEQ ID NO.: 191) to create SEQ ID NO.: 197. To be used to create a transcriptional fusion with β2AR-ToxR chimeras as shown in SEQ ID NO.: 205 and future GPCR-ToxR chimeras.

Oligonucleotides SEQ ID NOS.: 218, 219, 220 and 221 were used to amplify human Gα12/13 from human cDNA to create SEQ ID NO.: 196. Using XhoI and XbaI a translational fusion was made with ToxR residues 5-141 from Vibrio cholerae (SEQ ID NO.: 191) to create SEQ ID NO.: 201. To be used to create future transcriptional fusions with GPCR-ToxR chimeras as shown in SEQ ID NO.: 205.

Oligonucleotides SEQ ID NOS.: 222, 223, 224 and 225 were used to amplify human Gαq from human cDNA to create SEQ ID NO.: 194. Using XhoI and XbaI a translational fusion was made with ToxR residues 5-141 from Vibrio cholerae (SEQ ID NO.: 191) to create SEQ ID NO.: 199. To be used to create future transcriptional fusions with GPCR-ToxR chimeras as shown in SEQ ID NO.: 205.

Oligonucleotides SEQ ID NOS.: 226, 227, 228 and 229 were used to amplify human Giα from human cDNA to create SEQ ID NO.: 195. Using XhoI and XbaI a translational fusion was made with ToxR residues 5-141 from Vibrio cholerae (SEQ ID NO.: 191) to create SEQ ID NO.: 200. To be used to create future transcriptional fusions with GPCR-ToxR chimeras as shown in SEQ ID NO.: 205.

Oligonucleotides SEQ ID NOS.: 230, 231, 232 and 233 were used to amplify human GS2α from human cDNA to create SEQ ID NO.: 193. Using XhoI and XbaI a translational fusion was made with ToxR residues 5-141 from Vibrio cholerae (SEQ ID NO.: 191) to create SEQ ID NO.: 198. To be used to create future transcriptional fusions with GPCR-ToxR chimeras as shown in SEQ ID NO.: 205.

Oligonucleotides SEQ ID NOS.: 209, 210, 211 and 234 were used to amplify human β2AR from human cDNA to create SEQ ID NO.: 189. Using SalI and XhoI a transcriptional fusion was made between β2AR and human GS1α (SEQ ID NO.: 187) to create a SEQ ID NO.: 190. SEQ ID NO.: 190 was cloned into SEQ ID NOS.: 140, 142, 151 and 153 using SalI and XbaI.

Oligonucleotides SEQ ID NOS.: 236, 237, 238 and 239 were used to amplify bases coinciding with ToxR residues 5-141 from Vibrio Cholerae to create SEQ ID NO.: 202. Using PstI and XhoI a translational fusion was made between ToxR and human β2AR (SEQ ID NO.: 203) to create SEQ ID NO.: 204.

Oligonucleotides SEQ ID NOS.: 209, 210, 211 and 235 were used to amplify human β2AR from human cDNA to create SEQ ID NO.: 203. Using SalI and PstI a translational fusion was made between β2AR and ToxR (SEQ ID NO.: 202) to create SEQ ID NO.: 204.

Using oligonucleotides SEQ ID NOS.: 197 and 204 transcriptional fusions were created between the β2AR-ToxR translational fusion (SEQ ID NO.: 204) and the GS1α-ToxR translational fusion (SEQ ID NO.: 197) to create SEQ ID NO.: 205.

Oligonucleotides SEQ ID NOS.: 240, 241, 242 and 243 were used to amplify the ctx promoter region (Pctx) from Vibrio cholerae to create SEQ ID NO.: 206. Combining this PCR product in combination with the SEQ ID NO.: 207 PCR product and amplifying in the presence of SEQ ID NOS.: 242, 247, SEQ ID NO.: 208 was created. Using XbaI, the SEQ

ID NO.: 208 reporter construct was subsequently cloned into pACYC184 for co-transformation with the GPCR-G-protein fusions constructs above.

Oligonucleotides SEQ ID NOS.: 244, 245, 246 and 247 were used to amplify the lacZ from E. coli to create SEQ ID NO.: 207. Combining this PCR product in combination with the SEQ ID NO.: 206 PCR product and amplifying in the presence of SEQ ID NOS.: 242 and 247, SEQ ID NO.: 208 was created. Using XbaI, the 208 reporter construct was subsequently cloned into pACYC184 for co-transformation with the GPCR-G-protein fusions constructs above.

EXAMPLE 23. MODULAR MEMBRANE-TARGETING AND SOLUBILIZATION EXPRESSION CONSTRUCTS

To produce membrane proteins efficiently in minicells it may be necessary to create chimeric fusions with the membrane protein of interest. In this Example ,various regions of the MalE protein have been cloned into a modular expression system designed to create chimeric fusions with direct difficult to target membrane proteins to produce leader domains that will direct the proteins to the cytoplasmic membrane (Miller, K., W., et al. 1998. Production of active chimeric pediocin AcH in Escherichia coli in the absence of processing and secretion genes from the Pediococcus pap operon. Appl. Environ. Microbiol. 64:14-20). Similarly, a modified version of the TrxA protein has also been cloned into this modular expression system to create chimeric fusions with proteins that are difficult to maintain in a soluble conformation (LaVallie, E. R., et al. 1993. A thioredoxin gene fusion expression system that circumvents inclusion body formation in the E. coli cytoplasm. Biotechnology (N. Y.) 11:187-193). Table 31 describes each of these modular constructs.

TABLE 31. MODULAR MEMBRANE-TARGETING AND SOLUBILIZATION EXPRESSION CONSTRUCTS

Protein [a]	Construct [a]	SEQ ID NO
MalE (1-28)	NsiI-MalE(1-28)-Factor Xa-PstI, SalI, XbaI-FLAG, NheI	248
MalE (1-370, del 354-364)	NsiI-MalE(1-370, del 354-364)-Factor Xa-PstI, SalI, XbaI-FLAG, NheI	249
TrxA (2-109, del 103-107)	PstI, SalI, XbaI-TrxA(2-109, del 103-107)-FLAG-NheI	250

Protein [a]	Construct [a]	SEQ ID NO
MalE (1-28)-TrxA (2-109, del 103-107)	NsiI-MalE(1-28)-Factor Xa-PstI, SalI, XbaI-TrxA (2-109 del 103-107)-FLAG, NheI	251
MalE (1-370, del 354-364)-TrxA (2-109, del 103-107)	NsiI-MalE(1-370, del 354-364)-Factor Xa-PstI, SalI, XbaI-TrxA (2-109 del 103-107)-FLAG, NheI	252

a. The term "del" refers to a deletion in which amino acid residues following the term "del" are removed from the sequence.

TABLE 32. OLIGONUCLEOTIDE PRIMER SEQUENCES FOR TABLE 31.

SEQ ID NO.:	Primer name	5' to 3' sequence
253	MalE-1-NsiI	CGCGGATGCATATGAAAATAAAAACAGGTGCACGCAT CCTCGCATTATCCGCATTAACGACGATGATGTTTTCCG CCTCGGCTCTCGCC
254	MalE-2-middle	CGTCGACCGAGGCCTGCAGGCGGCTTCGATGATTTT GGCGAG AGCCGAGGCGGAAAACATCATCGTCG
255	MalE-3s-NheI	CGAAGCCCGCCTGCAGGCCTCGGTCGACGCCGAATCT AGAGATTATAAAGATGACGATGACAAATAATAAGCTA GCGGCGC
256	MalE-4-NheI	GCGCCGCTAGCTTATTATTTGTCATCG
257	MalE-1a	GGTGCACGCATCCTCGCATTATCCGC
258	MalE-2a	GGCGTTTTCCATGGTGGCGGCAATACGTGG
259	MalE-1-NsiI	CGCGGATGCATATGAAAATAAAAACAGGTGCACGCAT CCTC GCATTATCCGC
260	MalE-2-NheI	CCGAGGCCTGCAGGCGGCTTCGATACGCACGGCATA CCAG AAAGCGGACTGGGCGTTTTCCATGGTGGCGGCAATAC GTGG
261	MalE-3L-NheI	GCGCCGCTAGCTTATTATTTGTCATCGTCATCTTTATA ATCTC TAGATTCGGCGTCGACCGAGGCCTGCAGGCGGCTTC GATA CGC
262	TrxA-1a	CCTGACTGACGACAGTTTTGACACGG
263	TrxA-2a	CCTTTAGACAGTGCACCCACTTTGGTTGCCGC

SEQ ID NO.:	Primer name	5' to 3' sequence
264	TrxA-1a-PstI	CGCGGCTGCAGGCCTCGGTCGACGCCGAATCTAGAAGCGAT AAAATTATTCACCTGACTGACGACAGTTTTGACACGG
265	TrxA-2-NheI	GCGCCGCTAGCTTATTATTTGTCATCGTCATCTTTATAATCCG CCAGGTTCTCTTTCAACTGACCTTTAGACAGTGCACCCACTTT GGTTGCCGC

Oligonucleotides SEQ ID NOS.: 253, 254, 255 and 256 overlap with each other to form a scaffold template to PCR amplify malE (1-28) to create a SEQ ID NO.: 248. Following PCR amplification, SEQ ID NO.: 248 was digested with NsiI and NheI and cloned into SEQ ID NOS.: 152, 154, 139 and 141 digested with PstI and XbaI. The resultant products create SEQ ID NOS.: 266, 267, 268 and 269, respectively, that lose both the 5-prime PstI and 3-prime XbaI restriction site and retain the PstI, SalI, and XbaI restriction sites between MalE (1-28) and the FLAG sequence. Insertion of a protein in alignment with these sites results in a chimeric protein containing amino-terminal MalE (1-28) and carboxy-terminal FLAG.

Oligonucleotides SEQ ID NOS.: 257, 258, 259 and 260 were used to amplify malE (1-370 with a deletion removing residues 354-364) to create SEQ ID NO.: 249. Following PCR amplification, SEQ ID NO.: 249 was digested with NsiI and NheI and cloned into SEQ ID NOS.: 152, 154, 139 and 141 digested with PstI and XbaI. The resultant products create SEQ ID NOS.: 270, 271, 272 and 273, respectively, that lose both the 5-prime PstI and 3-prime XbaI restriction site and retain the PstI, SalI, and XbaI restriction sites between MalE (1-370, del 354-364) and the FLAG sequence. Insertion of a protein in alignment with these sites results in a chimeric protein containing amino-terminal MalE (1-370, del 354-364) and carboxy-terminal FLAG.

Oligonucleotides SEQ ID NOS.: 262, 263, 264 and 265 were used to amplify trxA (2-109 with a deletion removing residues 103-107) to create SEQ ID NO.: 250. Following PCR amplification, SEQ ID NO.: 250 was digested with PstI and NheI and cloned into SEQ ID NOS.: 152, 154, 139 and 141 digested with PstI and XbaI. to create SEQ ID NOS.: 274,

275, 276 and 277, respectively. Using these restriction digestion combinations results in loss of the XbaI SEQ ID NO.: 249 insertion site.

The resultant products create SEQ ID NOS.: 274, 275, 276 and 277, respectively, that lose the 3-prime XbaI restriction site and retain the PstI, SalI, and XbaI restriction sites on the 3-prime end of the TrxA (1-109, del 103-107) sequence. Insertion of a protein in alignment with these sites results in a chimeric protein containing Carboxy-terminal TrxA (1-109, del 103-107)-FLAG.

SEQ ID NO.: 248 was digested with NsiI and XbaI and cloned into SEQ ID NOS.: 274, 275, 276 and 277 that were digested with PstI and XbaI. The resultant products create SEQ ID NOS.: 278, 279, 280 and 281, respectively, that lose the 5 prime PstI restriction site and retain the PstI, SalI, and XbaI restriction sites between MalE (1-28) and TrxA (1-109, del 103-107). Insertion of a protein in alignment with these sites results in a chimeric protein containing amino-terminal MalE (1-28) and carboxy-terminal TrxA (1-109, del 103-107)-FLAG.

SEQ ID NO.: 249 was digested with NsiI and XbaI and cloned into SEQ ID NOS.: 274, 275, 276 and 277 that were digested with PstI and XbaI. The resultant products create SEQ ID NOS.: 282, 283, 284 and 285, respectively, that lose the 5 prime PstI restriction site and retain the PstI, SalI, and XbaI restriction sites between MalE (1-370, del 354-364) and TrxA (1-109, del 103-107). Insertion of a protein in alignment with these sites results in a chimeric protein containing amino-terminal MalE (1-370, del 354-364) and carboxy-terminal TrxA (1-109, del 103-107)-FLAG.

EXAMPLE 24: POROPLAST™ FORMATION

Minicells are used to prepare Poroplasts in order to increase the accessibility of a membrane protein component and/or domain to the outside environment. Membrane proteins in the inner membrane are accessible for ligand binding and/or other interactions in poroplasts, due to the absence of an outer membrane. The removal of the outer membrane from E. coli whole cells and minicells to produce poroplasts was carried out using modifications of previously described protoplast and analysis protocols (Birdsell et al., Production and Ultrastructure of Lysozyme and Ethylenediaminetetraacetate-Lysozyme Spheroplasts of Escherichia coli, J. Bacteriol. 93:427-437, 1967; Weiss et al., Protoplast

Formation in Escherichia Coli, J. Bacteriol. 128:668-670, 1976; Matsuyama, S-I., et al. SecD is involved in the release of translocated secretory proteins from the cytoplasmic membrane of Escherichia coli. 12:265-270, 1993).

In brief, cells were grown to late-log phase and pelleted at room temperature. Minicells were also isolated from cultures in late-log phase. The pellet was washed twice with 50 mM Tris, pH 8.0. Following the second wash, 1 X 10^9cells were resuspended in 1 ml 50 mM Tris (pH 8.0) that contained 8% sucrose and 2 mM EDTA. Cell/EDTA/sucrose mixtures were incubated at 37oC for 10 min, centrifuged, decanted, and poroplasted cells were resuspended in 50 mM Tris, pH 8.0 with 8% sucrose. Incubation with anti-LPS-coated magnetic beads, as described in Example 14, is used to enrich for poroplasts that lack LPS. Following incubation with the resuspended protoplasted cells, the anti-LPS magnetic beads were removed from suspension with a magnet.

To examine the range of molecular sizes that can pass through the cell wall, an IgG molecule was tested for its ability to pass the intact cell wall. Binding of an antibody to the ToxR-PhoA chimera expressed on the inner membrane minicell poroplasts was measured. Briefly, minicell poroplasts with and without inner membrane-bound ToxR-PhoA were incubated at 37°C with anti-PhoA antibody in reaction buffer (50 mM Tris, pH 8.0, 8% sucrose, 1% BSA, and 0.01% Tween-20). Following incubation, poroplasts were centrifuged, washed 3 times with reaction buffer, and resuspended in 50 mM Tris, pH 8.0 with 8% sucrose. Following resuspension, bound proteins from 5 X 10^7 minicells or minicell poroplasts were separated using denaturing SDS-PAGE, transferred to nitrocellulose, and developed using with both anti-PhoA antibody and secondary antibody against both heavy and light chains of anti-PhoA IgG (Table 33).

TABLE 33: ANTI-PHOA ACCESSIBILITY TO POROPLAST INNER MEMBRANE-BOUND TOXR-PHOA

EDTA (mM)	0	2	0	2
Lysozyme (mg/ml)	0	0	5	5
	Poroplasts (ng antibody bound)		Protoplasts (ng antibody bound)	

Minicells ToxR-PhoA	ND [a]	0.6	ND [a]	12.8
Minicells only	ND [a]	ND [a]	ND [a]	ND [a]

a. Non-detectable

These results demonstrate that the cell wall present on poroplasts is penetrable by an IgG molecule and that an IgG molecule is capable of passing the intact cell wall and binding to an inner membrane protein. From this data it appeats that poroplast operate at ~ 10% the efficiency of protoplasts by allowing 0.6 ng of IgG to bind inner membrane-bound ToxR-PhoA compared to 12.8 ng. However, given the large size of IgG (~150,000 Daltons) it is expected that molecules having a smaller molecular weight will efficiently access inner membrane proteins in poroplasts.

EXAMPLE 25: PRODUCTION OF NEUROTENSIN RECEPTOR (NTR).

To demonstrate expression of NTR in isolated minicells, MalE(L)-NTR (SEQ ID NO.: 166 was cloned into pMPX-67 (SEQ ID NO.: 151). Following minicell isolation, 1.5 X 10^9 minicells were induced with 1 mM Rhamnose for 2 hour at 37°C. Following induction, the protein produced was visualized via Western analysis using an anti-MalE antibody following separation on an SDS-PAGE. The results are shown in Figure 2.

These data demonstrates that MalE(L)-NTR is induced 87-fold by addition of 1 mM rhamnose to the minicell induction mixure. Cross-reactive proteins are host MalE and non-specific binding by Goat-anti-mouse HRP secondary antibody.

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The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents.

The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including," containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

1. A minicell comprising a membrane protein selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein and an organellar membrane protein.

2. The minicell of claim 1, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

3. The minicell of claim 1, wherein said minicell comprises a biologically active compound.

4. The minicell of claim 1, wherein said minicell comprises a expression construct, wherein said first expression construct comprises expression sequences operably linked to an ORF that encodes a protein.

5. The minicell of claim 4, wherein said ORF encodes said membrane protein.

6. The minicell of claim 4, wherein said expression sequences that are operably linked to an ORF are inducible and/or repressible.

7. The minicell of claim 4, wherein said minicell comprises a second expression construct, wherein said second expression construct comprises expression sequences operably linked to a gene.

8. The minicell of claim 7, wherein said expression sequences that are operably linked to a gene are inducible and/or repressible.

9. The minicell of claim 7, wherein the gene product of said gene regulates the expression of the ORF that encodes said protein.

10. The minicell of claim 7, wherein the gene product of said gene is a nucleic acid.

11. The minicell of claim 7, wherein the gene product of said gene is a polypeptide.

12. The minicell of claim 11, wherein said polypeptide is a membrane protein, a soluble protein or a secreted protein.

13. The minicell of claim 12, wherein said membrane protein is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide.

14. A minicell comprising a membrane fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide.

15. The minicell of claim 14, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

16. The minicell of claim 14, wherein said minicell comprises a biologically active compound.

17. A minicell comprising a membrane conjugate, wherein said membrane conjugate comprises a membrane protein chemically linked to a conjugated compound.

18. The minicell of claim 17, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

19. The minicell of claim 17, wherein said minicell comprises a biologically active compound.

20. The minicell of claim 17, wherein said conjugated compound is selected from the group consisting of a nucleic acid, a polypeptide, a lipid and a small molecule.

21. A method for making minicells, comprising

    (a) culturing a minicell-producing parent cell, wherein said parent cell comprises an expression construct, wherein said expression construct comprises a gene operably linked to expression sequences that are inducible and/or repressible, and wherein induction or repression of said gene causes or enhances the production of minicells; and

    (b) separating said minicells from said parent cell, thereby generating a composition comprising minicells,

    wherein an inducer or repressor is present within said parent cells during one or more steps and/or between two or more steps of said method.

22. The method of claim 21, further comprising

    (c) purifying said minicells from said composition.

23. The method of claim 21, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

24. The method of claim 21, wherein said gene expresses a gene product that is a factor that is involved in or modulates DNA replication, cellular division, cellular partitioning, septation, transcription, translation, or protein folding.

25. The method of claim 21, wherein said minicells are separated from said parent cells by a process selected from the group consisting of centrifugation, ultracentrifugation, density gradation, immunoaffinity and immunoprecipitation.

26. The method of claim 22, wherein said minicell is a poroplast, said method further comprising

(d) treating said minicells with an agent, or incubating said minicells under a set of conditions, that degrades the outer membrane of said minicell.

27. The method of claim 26, wherein said outer membrane is degraded by treatment with an agent selected from the group consisting of EDTA, EGTA, lactic acid, citric acid, gluconic acid, tartaric acid, polyethyleneimine, polycationic peptides, cationic leukocyte peptides, aminoglycosides, aminoglycosides, protamine, insect cecropins, reptilian magainins, polymers of basic amino acids, polymixin B, chloroform, nitrilotriacetic acid and sodium hexametaphosphate and/or by exposure to conditions selected from the group consisting of osmotic shock and insonation.

28. The method of claim 26, further comprising removing one or more contaminants from said composition.

29. The method of claim 28, wherein said contaminant is LPS or peptidoglycan.

30. The method of claim 29, wherein said LPS is removed by contacting said composition to an agent that binds or degrades LPS.

31. The method of claim 21, wherein said minicell-producing parent cell comprises a mutation in a gene required for lipopolysaccharide synthesis.

32. The method of claim 22, wherein said minicell is a spheroplast, said method further comprising

(d) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupts or degrades the outer membrane; and

(e) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupts or degrades the cell wall.

33. The method of claim 32, wherein said agent that disrupts or degrades the cell wall is a lysozyme, and said set of conditions that disrupts or degrades the cell wall is incubation in a hypertonic solution.

34. The method of claim 22, wherein said minicell is a protoplast, said method further comprising

(d) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupt or degrade the outer membrane;

(e) treating said minicells with an agent, or incubating said minicells under a set of conditions, that disrupts or degrades the cell wall, in order to generate a composition that comprises protoplasts; and

(f) purifying protoplasts from said composition.

35. The method of claim 22, further comprising preparing a denuded minicell from said minicell.

36. The method of claim 22, further comprising covalently or non-covalently linking one or more components of said minicell to a conjugated moiety.

37. A method of preparing a L-form minicell comprising:

(a) culturing an L-form eubacterium, wherein said eubacterium comprises one or more of the following:

(i) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene regulates the copy number of an episomal expression construct;

(ii) a mutation in an endogenous gene, wherein said mutation regulates the copy number of an episomal expression construct.

(iii) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene causes or enhances the production of minicells; and

(iv) a mutation in an endogenous gene, wherein said mutation causes or enhances minicell production.

(b) culturing said L-form minicell-producing parent cell in media under conditions wherein minicells are produced; and

(c) separating said minicells from said parent cell, thereby generating a composition comprising L-form minicells,

wherein an inducer or repressor is present within said minicells during one or more steps and/or between two or more steps of said method.

38. The method of claim 37, further comprising

(d) purifying said L-form minicells from said composition.

39. A method of producing a protein, comprising:

(a) transforming a minicell-producing parent cell with an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said protein;

(b) culturing said minicell-producing parent cell under conditions wherein minicells are produced; and

(c) purifying minicells from said parent cell,

(d) purifying said protein from said minicells.

wherein said ORF is expressed during step (b), between steps (b) and (c), and during step (c).

40. The method of claim 39, wherein said expression elements segregate into said minicells, and said ORF is expressed between steps (c) and (d).

41. The method of claim 39, wherein said protein is a membrane protein.

42. The method of claim 39, wherein said protein is a soluble protein contained within said minicells, further comprising:

(e) at least partially lysing said minicells.

43. The method of claim 39, wherein said protein is a secreted protein, wherein said method further comprises

(e) collecting a composition in which said minicells are suspended or with which said minicells are in contact.

44. The method of claim 39, wherein the expression sequences to which said ORF is operably linked are inducible, wherein said method further comprises adding an inducing agent between steps (a) and (b); during step (b); and between steps (b) and (c).

45. The method of claim 39, wherein the expression sequences to which said ORF is operably linked are inducible, wherein said expression elements segregate into said minicells, said method further comprises adding an inducing agent after step (c).

46. The method of claim 39, further comprising:

(e) preparing poroplasts from said minicells,

wherein said ORF is expressed during step (b); between steps (b) and (c); during step (c); between step (c) and step (d) when said expression elements segregate into said minicells; and/or after step (d) when said expression elements segregate into said minicells.

47. The method of claim 46, further comprising:

(f) purifying said protein from said poroplasts.

48. The method of claim 39, further comprising

(e) preparing spheroplasts from said minicells,

wherein said ORF is expressed during step (b), between steps (b) and (c), during step (c), between steps (c) and (d) and/or after step (d).

49. The method of claim 48, further comprising:

(f) purifying said protein from said spheroplasts.

50. The method of claim 39, further comprising

(e) preparing protoplasts from said minicells,

wherein said ORF is expressed during step (b), between steps (b) and (c), during step (c), between steps (c) and (d) and/or after step (d).

51. The method of claim 50, further comprising:

(f) purifying said protein from said protoplasts.

52. The method of claim 39, further comprising

(e) preparing membrane preparations from said minicells,

wherein said ORF is expressed during step (b), between steps (b) and (c), during step (c), between steps (c) and (d) and/or after step (d).

53. The method of claim 48, further comprising:

(f) purifying said protein from said membrane preparations.

54. The method of claim 39, wherein said minicell-producing parent cell is an L-form bacterium.

55. A method of producing a protein, comprising:

(a) transforming a minicell with an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said protein; and

(b) incubating said minicells under conditions wherein said ORF is expressed.

56. The method of claim 55, further comprising:

(c) purifying said protein from said minicells.

57. The method of claim 55, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

58. A method of producing a protein, comprising:

(a) transforming a minicell-producing parent cell with an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes a fusion protein comprising said protein and a polypeptide, wherein a protease-sensitive amino acid sequence is positioned between said protein and said polypeptide;

(b) culturing said minicell-producing parent cell under conditions wherein minicells are produced;

(c) purifying minicells from said parent cell, wherein said ORF is expressed during step (b); between steps (b) and (c); and/or after step (c) when said expression elements segregate into said minicells; and

(d) treating said minicells with a protease that cleaves said sensitive amino acid sequence, thereby separating said protein from said polypeptide.

59. A poroplast, said poroplast comprising a vesicle, bonded by a membrane, wherein said membrane is an eubacterial inner membrane, wherein said vesicle is surrounded by a eubacterial cell wall, and wherein said eubacterial inner membrane is accessible to a compound in solution with said poroplast.

60. The poroplast of claim 59, wherein said poroplast is a cellular poroplast.

61. The poroplast of claim 59, wherein said compound has a molecular weight of at least 1 kD.

62. The poroplast of claim 59, wherein said poroplast comprises an exogenous nucleic acid.

63. The poroplast of claim 62, wherein said exogenous nucleic acid is an expression construct.

64. The poroplast of claim 63, wherein said expression construct comprises an ORF that encodes an exogenous protein, wherein said ORF is operably linked to expression sequences.

65. The poroplast of claim 64, wherein said poroplast comprises an exogenous protein.

66. The poroplast of claim 59, wherein said poroplast comprises an exogenous protein.

67. The poroplast of claim 66, wherein said exogenous protein is a fusion protein, a soluble protein or a secreted protein.

68. The poroplast of claim 66, wherein said exogenous protein is a membrane protein.

69. The poroplast of claim 68, wherein said membrane protein is accessible to compounds in solution with said poroplast.

70. The poroplast of claim 68, wherein said membrane protein is selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein, and an organellar membrane protein.

71. The poroplast of claim 68, wherein said membrane protein is a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is displayed by said poroplast.

72. The poroplast of claim 71, wherein said second polypeptide is displayed on the external side of said eubacterial inner membrane.

73. The poroplast of claim 59, wherein said poroplast comprises a membrane component that is chemically linked to a conjugated compound.

74. The poroplast of claim 64, wherein said expression construct comprises one or more DNA fragments from a genome or cDNA.

75. The poroplast of claim 64, wherein said exogenous protein has a primary amino acid sequence that is predicted from in silico translation of a nucleic acid sequence.

76. A method of making poroplasts or cellular poroplasts, comprising treating eubacterial minicells or cells with an agent, or incubating said minicells or cells under a set of conditions, that degrades the outer membrane of said minicells or cells.

77. The method of claim 76, further comprising purifying said poroplasts or cellular poroplasts in order to remove contaminants.

78. The method of claim 76, further comprising placing said poroplasts in a hypertonic solution, wherein 90% or more of said cells or minicells used to prepare said poroplasts would lyse in said solution under the same conditions.

79. A solid support comprising a minicell.

80. The solid support of claim 79, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

81. The solid support of claim 79, wherein said solid support is a dipstick.

82. The solid support of claim 79, wherein said solid support is a bead.

83. The solid support of claim 79, wherein said solid support is a mictrotiter multiwell plate.

84. The solid support of claim 79, wherein said minicell comprises a detectable compound.

85. The solid support of claim 84, wherein said detectable compound is a fluorescent compound.

86. The solid support of claim 79, wherein said minicell displays a membrane component.

87. The solid support of claim 86, wherein said membrane component is selected from the group consisting of (i) a eukaryotic membrane protein, (ii) an archeabacterial membrane protein, (iii) an organellar membrane protein, (iv) a fusion protein comprising at least one transmembrane domain or at least one membrane anchoring domain, and (v) a membrane conjugate comprising a membrane component chemically linked to a conjugated compound.

88. The solid support of claim 86, wherein said membrane component is a receptor.

89. The solid support of claim 87, wherein said solid support further comprises a co-receptor.

90. The solid support of claim 79, wherein said minicell displays a binding moiety.

91. A solid support comprising a minicell, wherein said minicell displays a fusion protein, said fusion protein comprising a first polypeptide that comprises at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide.

92. The solid support of claim 91, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

93. The solid support of claim 91, wherein said second polypeptide comprises a binding moiety.

94. The solid support of claim 91, wherein said second polypeptide comprises an enzyme moiety.

95. A solid support comprising a minicell, wherein said minicell comprises a membrane conjugate comprising a membrane component chemically linked to a conjugated compound.

96. The solid support of claim 95, wherein said conjugated compound is a spacer.

97. The solid support of claim 96, wherein said spacer is covalently linked to said solid support.

98. The solid support of claim 95, wherein said conjugated compound is covalently linked to said solid support.

99. A minicell comprising a biologically active compound, wherein said minicell displays a binding moiety, wherein said binding moiety is part of a fusion protein comprising a first polypeptide that comprises at least one transmembrane domain or at least one

membrane anchoring domain and a second polypeptide that comprises a binding moiety, and said minicell is a poroplast, spheroplast or protoplast.

100. A eubacterial minicell comprising a biologically active compound, wherein said minicell displays a binding moiety, wherein said binding moiety is selected from the group consisting of (a) a eukaryotic membrane protein; (b) an archeabacterial membrane protein; (c) an organellar membrane protein; and (d) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety.

101. The minicell of claim 99, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a receptor and an active site of a non-catalytic derivative of an enzyme.

102. The minicell of claim 99, wherein said binding moiety is a single-chain antibody.

103. The minicell of claim 99, wherein said binding moiety is directed to a ligand selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

104. The minicell of claim 99, wherein said biologically active compound is selected from the group consisting of a radioisotope, a polypeptide, a nucleic acid and a small molecule.

105. The minicell of claim 99, further comprising a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

106. The minicell of claim 105, wherein one of said ORFs encodes a protein that comprises said binding moiety.

107. The minicell of claim 105, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

108. The minicell of claim 105, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

109. The minicell of claim 105, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

110. A method of associating a radioactive compound with a cell, wherein said cell displays a ligand specifically recognized by a binding moiety, comprising contacting said cell with a minicell that comprises said radioactive compound and displays said binding moiety.

111. The method of claim 110, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

112. The method of claim 110, wherein the amount of radiation emitted by said radioactive isotope is sufficient to be detectable.

113. The method of claim 110, wherein the amount of radiation emitted by said radioactive isotope is sufficient to be cytotoxic.

114. The method of claim 110, wherein said ligand displayed by said cell is selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

115. The method of claim 110, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a channel protein protein and a receptor.

116. The method of claim 110, wherein said binding moiety is a single-chain antibody.

117. The method of claim 110, wherein said binding moiety is selected from the group consisting of an aptamer and a small molecule.

118. A method of delivering a biologically active compound to a cell, wherein said cell displays a ligand specifically recognized by a binding moiety, comprising contacting said cell with a minicell that displays said binding moiety, wherein said minicell comprises said biologically active compound, and wherein the contents of said minicell are delivered into said cell from a minicell bound to said cell.

119. The method of claim 118, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

120. The method of claim 118, wherein said biologically active compound is selected from the group consisting of a nucleic acid, a lipid, a polypeptide, a radioactive compound, an ion and a small molecule.

121. The method of claim 118, wherein the membrane of said minicell comprises a system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell.

122. The method of claim 121, wherein said system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell is a Type III secretion system.

123. The method of claim 118, wherein said minicell further comprises a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

124. The method of claim 123, wherein one of said ORFs encodes a protein that comprises said binding moiety.

125. The method of claim 123, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

126. The method of claim 123, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

127. The method of claim 123, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

128. A minicell displaying a synthetic linking moiety, wherein said synthetic linking moiety is covalenty or non-covalently attached to a membrane component of said mincell.

129. The minicell of claim 128, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

130. A sterically stabilized minicell comprising a displayed moiety that has a longer half-life in vivo than a wild-type minicell, wherein said displayed moiety is a hydrophilic polymer that comprises a PEG moiety, a carboxylic group of a polyalkylene glycol or PEG stearate.

131. A minicell having a membrane comprising an exogenous lipid, wherein a minicell comprising said exogenous lipid has a longer half-life in vivo than a minicell lacking said exogenous lipid, and wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

132. The minicell of claim 131, wherein said exogenous lipid is a derivitized lipid.

133. The minicell of claim 132, wherein said derivitized lipid is selected from the group consisting of phosphatidylethanolamine derivatized with PEG, DSPE-PEG, PEG stearate; PEG-derivatized phospholipids, and PEG ceramides is DSPE-PEG.

134. The minicell of claim 131, wherein said exogenous lipid is not present in a wild-type membrane, or is present in a different proportion than is found in minicells comprising a wild-type membrane,

135. The minicell of claim 134, wherein said exogenous lipid is selected from the group consisting of ganglioside, sphingomyelin, monosialoganglioside GM1, galactocerebroside sulfate, 1,2-sn-dimyristoylphosphatidylcholine, phosphatidylinositol and cardiolipin.

136. The minicell of claim 128, wherein said linking moiety is non-covalently attached to said minicell.

137. The minicell of claim 136, wherein one of said linking moiety and said membrane component comprises biotin, and the other comprises avidin or streptavidin.

138. The minicell of claim 128, wherein said synthetic linking moiety is a cross-linker.

139. The minicell of claim 128, wherein said cross-linker is a bifunctional cross-linker.

140. A method of transferring a membrane protein from a minicell membrane to a biological membrane comprising contacting a minicell to said biological membrane, wherein said minicell membrane comprises said membrane protein, and allowing said mincell and said biological membrane to remain in contact for a period of time sufficient for said transfer to occur.

141. The method of claim 140, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

142. The method of claim 140, wherein biological membrane is a cytoplasmic membrane or an organellar membrane.

143. The method of claim 140, wherein said biological membrane is a membrane selected from the group consisting of a membrane of a pathogen, a membrane of an infected cell and a membrane of a hyperproliferative cell.

144. The method of claim 140, wherein said biological membrane is the cytoplasmic membrane of a recipient cell.

145. The method of claim 144, wherein said recipient cell is selected from the group consisting of a cultured cell and a cell within an organism.

146. The method of claim 140, wherein biological membrane is present on a cell that has been removed from an animal, said contacting occurs in vitro, after which said cell is returned to said organism.

147. The method of claim 144, wherein said membrane protein is an enzyme.

148. The method of claim 147, wherein said membrane protein having enzymatic activity is selected from the group consisting of a cytochrome P450 and a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one polypeptide, wherein said second polypeptide has enzymatic acitivity.

149. The method of claim 140, wherein said membrane protein is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide.

150. The method of claim 149, wherein said second polypeptide is a biologically active polypeptide.

151. The method of claim 140, wherein said minicell displays a binding moiety.

152. A minicell that comprises an expression construct comprising an ORF encoding a membrane protein operably linked to expression sequences, wherein said expression sequences are induced and/or derepressed when said minicell is in contact with a target cell.

153. The minicell of claim 152, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

154. The minicell of claim 152, wherein biological membrane is a cytoplasmic membrane or an organellar membrane.

155. The minicell of claim 152, wherein said biological membrane is a membrane selected from the group consisting of a membrane of a pathogen, a membrane of an infected cell and a membrane of a hyperproliferative cell.

156. The minicell of claim 152, wherein said minicell displays a binding moiety.

157. The minicell of claim 156, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, an aptamer and a small molecule.

158. The minicell of claim 152, wherein said membrane protein is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide.

159. The minicell of claim 152, wherein said membrane protein having enzymatic activity is selected from the group consisting of a cytochrome P450 and a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one polypeptide, wherein said second polypeptide has enzymatic activity.

160. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein, wherein said membrane protein is selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein and an organellar membrane protein.

161. The pharmaceutical composition of claim 160, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

162. The pharmaceutical composition of claim 160, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein, a cellular adhesion factor and an integrin.

163. The pharmaceutical formulation of claim 162, wherein said pharmaceutical formulation further comprises an adjuvant.

164. The pharmaceutical formulation of claim 162, wherein said membrane protein comprises a polypeptide epitope displayed by a hyperproliferative cell.

165. The pharmaceutical formulation of claim 162, wherein said membrane protein comprises an epitope displayed by a eukaryotic pathogen, an archeabacterial pathogen, a virus or an infected cell.

166. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein that is a fusion protein, said fusion protein comprising (i) a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and (ii) a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein.

167. The pharmaceutical composition of claim 166, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

168. The pharmaceutical formulation of claim 167, wherein said pharmaceutical formulation further comprises an adjuvant.

169. The pharmaceutical formulation of claim 167, wherein said second polypeptide comprises a polypeptide epitope displayed by a hyperproliferative cell.

170. The pharmaceutical formulation of claim 169, wherein said membrane protein comprises an epitope displayed by a eukaryotic pathogen, an archeabacterial pathogen, a virus or an infected cell.

171. A pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane conjugate, wherein said membrane conjugate comprises a membrane component chemically linked to a conjugated compound.

172. The pharmaceutical composition of claim 171, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

173. The pharmaceutical composition of claim 171, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein, a cellular adhesion factor and an integrin.

174. The pharmaceutical composition of claim 171, wherein said pharmaceutical further comprises an adjuvant.

175. The pharmaceutical composition of claim 171, wherein said membrane component is a polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, or a lipid that is part of a membrane.

176. The pharmaceutical composition of claim 171, wherein said conjugated compound is a polypeptide, and the chemical linkage between said membrane compound and said conjugated compound is not a peptide bond.

177. The pharmaceutical composition of claim 171, wherein said conjugated compound is a nucleic acid.

178. The pharmaceutical composition of claim 171, wherein said conjugated compound is an organic compound.

179. The pharmaceutical composition of claim 176, wherein said organic compound is selected from the group consisting of a narcotic, a toxin, a venom, a sphingolipid and a soluble protein.

180. A method of making a pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein, wherein said membrane protein is selected from the group consisting of a eukaryotic membrane protein, an archeabacterial membrane protein and an organellar membrane protein.

181. The method of claim 1, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

182. The method of claim 180, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

183. The method of claim 180, wherein said method further comprises desiccating said formulation.

184. The method of claim 183, wherein said method further comprises adding a suspension buffer to said formulation.

185. The method of claim 180, wherein said method further comprises making a chemical modification of said membrane protein.

186. The method of claim 185, wherein said chemical modification is selected from the group consisting of glycosylation, deglycosylation, phosphorylation, dephosphorylation and proteolysis.

187 A method of making a pharmaceutical composition comprising a minicell, wherein said minicell displays a membrane protein that is a fusion protein, said fusion protein comprising (i) a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and (ii) a second

polypeptide, wherein said second polypeptide is not derived from a eubacterial protein.

188. The method of claim 187, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

189. The method of claim 187, wherein said method further comprises desiccating said pharmaceutical formulation.

190. The method of claim 189 wherein said method further comprises adding a suspension buffer to said pharmaceutical formulation.

191. The method of claim 187, wherein said method further comprises making a chemical modification of said membrane protein.

192. The method of claim 191, wherein said chemical modification is selected from the group consisting of glycosylation, deglycosylation, phosphorylation, dephosphorylation and proteolysis.

193. A method of making a pharmaceutical formulation comprising a minicell, wherein said minicell displays a membrane conjugate, wherein said membrane conjugate comprises a membrane component chemically linked to a conjugated compound.

194. The method of claim 193, wherein said method further comprises adding an adjuvant to said pharmaceutical formulation.

195. The method of claim 193, wherein said membrane component is a polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, or a lipid that is part of a membrane.

196. The method of claim 193, wherein said conjugated compound is a polypeptide, and the chemical linkage between said membrane compound and said conjugated compound is not a peptide bond.

197. The method of claim 193, wherein said conjugated compound is a nucleic acid.

198. The method of claim 193, wherein said conjugated compound is an organic compound.

199. The method of claim 186, wherein said organic compound is selected from the group consisting of a narcotic, a toxin, a venom, and a sphingolipid.

200. A method of detecting an agent that is specifically bound by a binding moiety, comprising contacting a minicell displaying said binding moiety with a composition known or suspected to contain said agent, and detecting a signal that is modulated by the binding of said agent to said binding moiety.

201. The method of claim 200, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

202. The method of claim 200, wherein said agent is associated with a disease.

203. The method of claim 200, wherein said minicell comprises a detectable compound.

204. The method of claim 200, wherein said binding moiety is antibody or antibody derivative.

205. The method of claim 200, wherein said composition is an environmental sample.

206. The method of claim 200, wherein said composition is a biological sample.

207. The method of claim 206, wherein said biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, a biopsy sample, feces and a skin patch.

208. A method of in situ imaging of a tissue or organ, comprising administering to an organism a minicell comprising an imaging agent and a binding moiety and detecting said imaging agent in said organism.

209. The method of claim 208, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

210. The method of claim 208, wherein said binding moiety is an antibody or antibody derivative.

211. The method of claim 208, wherein said binding moiety specifically binds a cell surface antigen.

212. The method of claim 211, wherein said cell surface antigen is an antigen displayed by a tumorigenic cell, a cancer cell, and an infected cell.

213. The method of claim 211, wherein said cell surface antigen is a tissue-specific antigen.

214. The method of claim208, wherein said method of imaging is selected from the group consisting of magnetic resonance imaging, ultrasound imaging; and computer axaial tomography (CAT).

215. A device comprising a microchip operatively associated with a biosensor comprising a minicell, wherein said microchip comprises or contacts said minicell, and wherein said minicell displays a binding moiety.

216. The device of claim 215, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

217. A method of detecting a substance that is specifically bound by a binding moiety, comprising contacting the device of claim 215 with a composition known or suspected to contain said substance, and detecting a signal from said device, wherein said signal changes as a function of the amount of said substance present in said composition.

218. The method of claim 217, wherein said composition is a biological sample or an environmental sample.

219 A method of identifying an agent that specifically binds a target compound, comprising contacting a minicell displaying said target compound with a library of compounds, and identifying an agent in said library that binds said target compound.

220. The method of claim 219, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

221. The method of claim 219, wherein said library of compounds is a protein library.

222. The method of claim 221, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, a baculovirus library, a yeast display library, and a ribosomal display library.

223. The method of claim 219, wherein said library of compounds is selected from the group consisting of a library of aptamers, a library of synthetic peptides and a library of small molecules.

224. The method of claim 219, wherein said target compound is a target polypeptide.

225. The method of claim 224, wherein said minicell comprises an expression construct comprising expression sequences operably linked to an ORF encoding said target polypeptide.

226. The method of claim 224, wherein said target polypeptide is a membrane protein.

227. The method of claim 226, wherein said membrane protein is a receptor or a channel protein.

228. The method of claim 226, wherein said membrane protein is an enzyme.

229. The method of claim 219, wherein said target compound is a membrane fusion protein, said membrane fusion protein comprising a first polypeptide, wherein said first polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide comprises amino acid sequences derived from a target polypeptide.

230. The method of claim 219, wherein said method further comprises comparing the activity of said target compound in the presence of said agent to the activity of said target compound in the absence of said agent.

231. The method of claim 230, wherein said activity of said target compound is an enzyme activity.

232. The method of claim 231, wherein said enzyme is selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

233. The method of claim 230, wherein said activity of said target compound is a binding activity.

234. The method of claim 233, further comprising comparing the binding of said agent to said target compound to the binding of a known ligand of said target compound.

235. The method of claim 234, wherein a competition assay is used for said comparing.

236. A device comprising microchips operatively associated with a biosensor comprising a set of minicells in a prearranged pattern, wherein said each of said microchips comprise or contact a minicell, wherein each of said minicell displays a different target compound, and wherein binding of a ligand to a target compound results in an increased or decreased signal.

237. A method of identifying an agent that specifically binds a target compound, comprising contacting the device of claim 236 with a library of compounds, and detecting a signal from said device, wherein said signal changes as a function of the binding of an agent to said target compound.

238. A method of identifying an agent that specifically blocks the binding of a target compound to its ligand, comprising contacting the device of claim 236 with a library of compounds, and detecting a signal from said device, wherein said signal changes as a function of the binding of an agent to said target compound.

239. A method of making a antibody that specifically binds a protein domain, wherein said domain is in its native conformation, wherein said domain is contained within a protein displayed on a minicell, comprising contacting said minicell with a cell, wherein said cell is competent for producing antibodies to an antigen contacted with said cell, in order to generate an immunogenic response in which said cell produces said antibody.

240. The method of claim 239, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

241. The method of claim 239, wherein said protein displayed on a minicell is a membrane protein.

242. The method of claim 241, wherein said membrane protein is a receptor or a channel protein.

243. The method of claim 239, wherein said domain is found within the second polypeptide of a membrane fusion protein, wherein said membrane fusion protein comprises a first polypeptide, wherein said first polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain.

244. The method of claim 239, wherein said contacting occurs in vivo.

245. The method of claim 244, wherein said antibody is a polyclonal antibody or a monoclonal antibody.

246. The method of claim 244, wherein said contacting occurs in an animal that comprises an adjuvant.

247. The method of making an antibody derivative that specifically binds a protein domain, wherein said domain is in its native conformation, wherein said domain is displayed on a minicell, comprising contacting said minicell with a protein library, and identifying an antibody derivative from said protein library that specifically binds said protein domain.

248. The method of claim 247, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

249. The method of claim 247 wherein said antibody derivative is a single-chain antibody.

250. A method of making an antibody or antibody derivative that specifically binds an epitope, wherein said epitope is selected from the group consisting of (i) an epitope composed of amino acids found within a membrane protein, (ii) an epitope present in an interface between a membrane protein and a membrane component, (iii) an epitope present in an interface between a membrane protein and one or more other proteins and (iv) an epitope in a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, said second polypeptide comprising said epitope; comprising contacting a minicell displaying said epitope with a protein library, or to a cell, wherein said cell is competent for producing antibodies to an antigen contacted with said cell, in order to generate an immunogenic response in which said cell produces said antibody.

251. The method of claim 250, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

252. The method of claim 250, wherein said cell is contacted in vivo.

253. The method of claim 252, wherein said antibody is a polyclonal antibody.

254. The method of claim 252, wherein said antibody is a monoclonal antibody.

255. The method of claim 250, wherein said protein library is contacted in vitro.

256. The method of claim 255, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

257. The method of claim 256, wherein said antibody derivative is a single-chain antibody.

258. A method of determining the rate of transfer of nucleic acid from a minicell to a cell, comprising

(a) contacting said cell to said minicell, wherein said minicell comprises said nucleic acid, for a set period of time;

(b) separating minicells from said cells;

(c) measuring the amount of nucleic acid in said cells,

wherein the amount of nucleic acid in said cells over said set period of time is the rate of transfer of a nucleic acid from a minicell.

259. A method of determining the amount of a nucleic acid transferred to a cell from a minicell, comprising

(a) contacting said cell to said minicell, wherein said minicell comprises an expression element having eukaryotic expression sequences operably linked to an ORF encoding a detectable polypeptide, wherein said minicell displays a binding moiety, and wherein said binding moiety binds an epitope of said cell; and

(b) detecting a signal from said detectable polypeptide,

wherein a change in said signal corresponds to an increase in the amount of a nucleic acid transferred to a cell.

260. The method of claim 258, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

261. The method of claim 258, wherein said cell is a eukaryotic cell.

262. The method of claim 258, wherein said binding moiety is an antibody or antibody derivative.

263. The method of claim 258, wherein said binding moiety is a single-chain antibody.

264. The method of claim 258, wherein said binding moiety is an aptamer.

265. The method of claim 258, wherein said binding moiety is an organic compound.

266. The method of claim 258, wherein said detectable polypeptide is a fluorescent polypeptide.

267. A method of detecting the expression of an expression element in a cell, comprising

(a) contacting said cell to a minicell, wherein said minicell comprises an expression element having cellular expression sequences operably linked to an ORF encoding a detectable polypeptide, wherein said minicell displays a binding moiety, and wherein said binding moiety binds an epitope of said cell;

(b) incubating said cell and said minicell for a period of time effective for transfer of nucleic acid from said minicell to said cell; and

(c) detecting a signal from said detectable polypeptide,

wherein an increase in said signal corresponds to an increase in the expression of said expression element.

268. The method of claim 267, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

269. The method of claim 267, wherein said cell is a eukaryotic cell and said expression sequences are eukaryotic expression sequences.

270. The method of claim 269, wherein said eukaryotic cell is a mammalian cell.

271. The method of claim267, wherein said binding moiety is an antibody or antibody derivative.

272. The method of claim 267, wherein said binding moiety is a single-chain antibody.

273. The method of claim 267, wherein said binding moiety is an aptamer.

274. The method of claim 267, wherein said binding moiety is an organic compound.

275. The method of claim 267, wherein said detectable polypeptide is a fluorescent polypeptide.

276. A method for detecting the transfer of a fusion protein from the cytosol to an organelle of a eukaryotic cell, comprising

(a) contacting said cell to a minicell, wherein

(i) said minicell comprises an expression element having eukaryotic expression sequences operably linked to an ORF encoding a fusion protein, wherein said fusion protein comprises a first polypeptide that comprises organellar delivery sequences, and a second polypeptide that comprises a detectable polypeptide; and

(ii) said minicell displays a binding moiety that binds an epitope of said cell, or an epitope of an organelle;

(b) incubating said cell and said minicell for a period of time effective for transfer of nucleic acid from said minicell to said cell and production of said fusion protein; and

(c) detecting a signal from the detectable polypeptide,

wherein a change in the signal corresponds to an increase in the amount of the fusion protein transferred to said organelle.

277. The method of claim 276, wherein said organelle is a mitochondrion, a chloroplast or a kinetoplast.

278. A minicell comprising at least one nucleic acid, wherein said minicell displays a binding moiety directed to a target compound, wherein said binding moiety is selected from the group consisting of (i) a eukaryotic membrane protein; (ii) an archeabacterial membrane protein; (iii) an organellar membrane protein; and (iv) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety.

279. The minicell of claim 278, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

280. The minicell of claim 278, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF encoding a protein selected from the group consisting of (i) said eukaryotic membrane protein, (ii) said archeabacterial membrane protein, (iii) said organellar membrane protein; and (iv) said fusion protein.

281. The minicell of claim 280, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a therapeutic polypeptide.

282. The minicell of claim 281, wherein said therapeutic polypeptide is a membrane polypeptide.

283. The minicell of claim 281, wherein said therapeutic polypeptide is a soluble polypeptide.

284. The minicell of claim 283, wherein said soluble polypeptide comprises a cellular secretion sequence.

285. The minicell of claim 281, wherein said expression sequences are inducible and/or repressible.

286. The minicell of claim2858, wherein said expression sequences are induced and/or derepressed when the binding moiety displayed by said minicell binds to its target compound.

287. The minicell of claim 1278herein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of a biologically active compound contained within or displayed by said minicell.

288 The minicell of claim 278 wherein the membrane of said minicell comprises a system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell.

289 The minicell of claim 288 wherein said system for transferring a molecule from the interior of a minicell into the cytoplasm of said cell is a Type III secretion system.

290. A method of introducing a nucleic acid into a cell, comprising contacting said cell with a minicell that comprises said nucleic acid, wherein said minicell displays a binding moiety, wherein said binding moiety is selected from the group consisting of (i) a eukaryotic membrane protein; (ii) an archeabacterial membrane protein; (iii) an organellar membrane protein; and (iv) a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain; and a second polypeptide, wherein said second polypeptide is not derived from a eubacterial protein and is neither a His tag nor a glutathione-S-transferase polypeptide, and wherein said polypeptide comprises a binding moiety; and wherein said binding moiety binds an epitope of said cell.

291. The method of claim 290, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

292. The method of claim 290, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF encoding a protein selected from the group consisting of (i) said eukaryotic membrane protein,

(ii) said archeabacterial membrane protein, (iii) said organellar membrane protein; and (iv) said fusion protein.

293. The method of claim 290, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a therapeutic polypeptide.

294. The method of claim 293, wherein said expression sequences are inducible and/or derepressible.

295. The method of claim 294, wherein said expression sequences are induced or derepressed when the binding moiety displayed by said minicell binds its target compound.

296. The method of claim 294, wherein said expression sequences are induced or derepressed by a transactivation or transrepression event.

297. The method of claim 292, wherein said nucleic acid comprises an expression construct comprising expression sequences operably linked to an ORF, wherein said ORF encodes a polypeptide having an amino acid sequence that facilitates cellular transfer of a biologically active compound contained within or displayed by said minicell.

298. A minicell comprising a nucleic acid, wherein said nucleic acid comprises eukaryotic expression sequences and eubacterial expression sequences, each of which is independently operably linked to an ORF.

299. The minicell of claim 298, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

300. The minicell of claim 298, wherein said minicell displays a binding moiety.

301. The minicell of claim 300, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

302. The minicell of claim 300, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

303. The minicell of claim 301, wherein the protein encoded by said ORF comprises eubacterial or eukaryotic secretion sequences.

304. A minicell comprising a first and second nucleic acid, wherein said first nucleic acid comprises eukaryotic expression sequences operably linked to a first ORF, and a

second nucleic acid, wherein said second nucleic acid comprises eubacterial expression sequences operably linked to a second ORF.

305. The minicell of claim 304, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

306. The minicell of claim 304, wherein said minicell displays a binding moiety.

307. The minicell of claim 306, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

308. The minicell of claim 306, wherein said eukaryotic expression sequences are induced and/or derepressed when said nucleic acid is in the cytoplasm of a eukaryotic cell.

309. The minicell of claim 304, wherein the protein encoded by said first ORF comprises eukaryotic secretion sequences and/or the protein encoded by said second ORF comprises eubacterial secretion sequences.

310. A method of introducing into and expressing a nucleic acid in an organism, comprising contacting a minicell to a cell of said organism, wherein said minicell comprises said nucleic acid.

311. The method of claim 310, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

312. The method of claim 310, wherein said minicell displays a binding moiety.

313. The method of claim 310, wherein said nucleic acid comprises a eukaryotic expression construct, wherein said eukaryotic expression construct comprises eukaryotic expression sequences operably linked to an ORF.

314. The method of claim 310, wherein said ORF encodes a protein selected from the group consisting of a membrane protein, a soluble protein and a protein comprising eukaryotic secretion signal sequences.

315. The method of claim 310, wherein said nucleic acid comprises a eubacterial expression construct, wherein said eubacterial expression construct comprises eubacterial expression sequences operably linked to an ORF.

316. The method of claim 315, wherein said minicell displays a binding moiety, wherein said eubacterial expression sequences are induced and/or derepressed when said binding moiety is in contact with a target cell.

317. The method of claim 316, wherein the protein encoded by said ORF comprises eubacterial secretion sequences.

318. A minicell comprising a crystal of a membrane protein.

319. The minicell of claim 318, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

320. The minicell of claim 318, wherein said membrane protein is a receptor.

321. The minicell of claim 320, wherein said receptor is a G-protein coupled receptor.

322. The minicell of claim 318, wherein said crystal is displayed.

323. The minicell of claim 318, wherein said membrane protein is a fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide.

324. The minicell of claim 323, wherein said crystal is a crystal of said second polypeptide.

325. The minicell of claim 323, wherein said crystal is displayed.

326. A method of determining the three-dimensional structure of a membrane protein, comprising preparing a crystal of said membrane protein in a minicell, and determining the three-dimensional structure of said crystal.

327. A method for identifying ligand-interacting atoms in a defined three-dimensional structure of a target protein, comprising (a) preparing one or more variant proteins of a target protein having a known or predicted three-dimensional structure, wherein said target protein binds a preselected ligand; (b) expressing and displaying a variant protein in a minicell; and (c) determining if a minicell displaying said variant protein binds said preselected ligand with increased or decreased affinity as compared to the binding of said preselected ligand to said target protein.

328. The method of claim 327, wherein said ligand is a protein that forms a multimer with said target protein, and said ligand interacting atoms are atoms in said defined three-dimensional structure are atoms that are involved in protein-protein interactions.

329. The method of claim 327, wherein said ligand is a compound that induces a conformational change in said target protein, and said defined three-dimensional structure is the site of said conformational change.

330. The method of claim 327, adopted to a method, said method for identifying ligands of a target protein, further comprising identifying the chemical differences in said variant proteins as compared to said target protein.

331. The method of claim 330, further comprising mapping said chemical differences onto said defined three-dimensional structure, and correlating the effect of said chemical differences on said defined three-dimensional structure.

332. The method of claim 331, wherein said target protein is a wild-type protein.

333. A minicell library, comprising two or more minicells, wherein each minicell comprises a different exogenous protein.

334. The minicell library of claim 333, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

335. The minicell library of claim 333, wherein said exogenous protein is a displayed protein.

336. The minicell library of claim 333, wherein said exogenous protein is a membrane protein.

337. The minicell library of claim 336, wherein said membrane protein is a receptor.

338. The minicell library of claim 333, wherein said protein is a soluble protein that is contained within or secreted from said minicell.

339. The minicell library of claim 333, wherein minicells within said library comprise an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said exogenous protein.

340. The minicell library of claim 339, wherein said nucleic acid has been mutagenized.

341. The minicell library of claim 339, wherein an active site of said exogenous protein has a known or predicted three-dimensional structure, and said a portion of said ORF encoding said active site has been mutagenized.

342. The minicell library of claim 333, wherein each of said minicells comprises an exogenous protein that is a variant of a protein having a known or predicted three-dimensional structure.

343. A minicell library, comprising two or more minicells, wherein each minicell comprises a different fusion protein, each of said fusion protein comprising a first

polypeptide that is a constant polypeptide, wherein said constant polypeptide comprises at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, wherein said second polypeptide is a variable amino acid sequence that is different in each fusion proteins.

344. The minicell library of claim 343, wherein minicells within said library comprise an expression element that comprises expression sequences operably linked to a nucleic acid having an ORF that encodes said fusion protein.

345. The minicell library of claim 344, wherein said second polypeptide of said fusion protein is encoded by a nucleic acid that has been cloned.

346. The minicell library of claim 344, wherein each of said second polypeptide of each of said fusion proteins comprises a variant of an amino acid sequence from a protein having a known or predicted three-dimensional structure.

347. A minicell library, comprising two or more minicells, wherein each minicell comprises a constant protein that is present in each minicell and a variable protein that differs from minicell to minicell.

348. The minicell library of claim 347, wherein one of said constant and variable proteins is a receptor, and the other of said constant and variable proteins is a co-receptor.

349. The minicell library of claim 347, wherein each of said constant and variable proteins is different from each other and is a factor in a signal transduction pathway.

350. The minicell library of claim 347, wherein one of said constant and variable proteins is a G-protein, and the other of said constant and variable proteins is a G-protein coupled receptor.

351. The minicell library of claim 347, wherein one of said constant and variable proteins comprises a first transrepression domain, and the other of said constant and variable comprises a second transrepression domain, wherein said transrepression domains limit or block expression of a reporter gene when said constant and variable proteins associate with each other.

352. The minicell library of claim 347, wherein one of said constant and variable proteins comprises a first transactivation domain, and the other of said constant and variable comprises a second transactivation domain, wherein said transactivation domains stimulate expression of a reporter gene when said constant and variable proteins associate with each other.

353. A method of identifying a nucleic acid that encodes a protein that binds to or chemically alters a preselected ligand, comprising:

(a) separately contacting said ligand with individual members of a minicell library, wherein minicells in said library comprise expression elements, wherein said expression elements comprise DNA inserts, wherein an ORF in said DNA insert is operably linked to expression sequences, in order to generate a series of reaction mixes, each reaction mix comprising a different member of said minicell library;

(b) incubating said reaction mixes, thereby allowing a protein that binds to or chemically alters said preselected ligand to bind or chemically alter said preselected ligand;

(c) detecting a change in a signal from reaction mixes in which said ligand has been bound or chemically altered;

(d) preparing DNA from reaction mixes in which said ligand has been bound or chemically altered;

wherein said DNA is a nucleic acid that encodes a protein that binds to or chemically alters said preselected ligand.

354. The method of claim 353, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

355. The method of claim 353, wherein said preselected ligand is a biologically active compound.

356. The method of claim 353, wherein said preselected ligand is a therapeutic drug.

357. The method of claim 353, wherein a protein that binds or chemically alters said preselected ligand is a target protein for compounds that are therapeutic for a disease that is treated by administering said drug to an organism in need thereof.

358. The method of claim 353, wherein said preselected ligand is detectably labeled, said mincell comprises a detectable compound, and/or a chemically altered derivative of said protein is detectably labeled.

359. A method of determining the amino acid sequence of a protein that binds or chemically alters a preselected ligand, comprising:

(a) contacting said ligand with a minicell library, wherein minicells in said library comprise expression elements, wherein said expression elements comprise DNA inserts, wherein an ORF in said DNA insert is operably linked to expression sequences;

(b) incubating said mixture of ligand and minicells, under conditions which allow complexes comprising ligands and minicells to form and/or chemical reactions to occur;

(c) isolating or identifying said complexes from said ligand and said mixture of ligand and minicells;

(d) preparing DNA from an expression element found in one or more of said complexes, or in a minicell thereof;

(e) determining the nucleotide sequence of said ORF in said DNA; and

(f) generating an amino sequence by in silico translation, wherein said amino acid sequence is or is derived from a protein that binds or chemically alters a preselected ligand.

360. The method of claim 359, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

361. The method of claim 359, wherein said DNA is prepared by isolating DNA from said complexes, or in a minicell thereof.

362. The method of claim 359, wherein said DNA is prepared by amplifying DNA from said complexes, or in a minicell thereof.

363. The method of claim 359, wherein said protein is a fusion protein.

364. The method of claim 359, wherein said protein is a membrane or a soluble protein.

365. The method of claim 364, wherein said protein comprises secretion sequences.

366. The method of claim 359, wherein said preselected ligand is a biologically active compound.

367. The method of claim 359, wherein said preselected ligand is a therapeutic drug.

368. The method of claim 359, wherein said preselected ligand is a therapeutic drug, and said protein that binds said preselected ligand is a target protein for compounds that

are therapeutic for a disease that is treated by administering said drug to an organism in need thereof.

369. A method of identifying a nucleic acid that encodes a protein that inhibits or blocks an agent from binding to or chemically altering a preselected ligand, comprising:

(a) separately contacting said ligand with individual members of a minicell library, wherein minicells in said library comprise expression elements, wherein said expression elements comprise DNA inserts, wherein an ORF in said DNA insert is operably linked to expression sequences, in order to generate a series of reaction mixes, each reaction mix comprising a different member of said minicell library;

(b) incubating said reaction mixes, thereby allowing a protein that binds to or chemically alters said preselected ligand to bind or chemically alter said preselected ligand;

(c) detecting a change in a signal from reaction mixes in which said ligand has been bound or chemically altered;

(d) preparing DNA from reaction mixes in which said change in signal ligand has been bound or chemically altered;

wherein said DNA is a nucleic acid that encodes a protein that inhibits or blocks said agent from binding to or chemically altering said preselected ligand

370. The method of claim 369, wherein said minicell is a eubacterial minicell, a poroplast, a spheroplast or a protoplast.

371. The method of claim 369, wherein said DNA has a nucleotide sequence that encodes the amino acid sequence of said protein that inhibits or blocks said agent from binding to or chemically altering said preselected ligand.

372. The method of claim 369, wherein a protein that binds or chemically alters said preselected ligand is a target protein for compounds that are therapeutic for a disease that is treated by administering said drug to an organism in need thereof.

373. A method of identifying an agent that effects the activity of a protein, comprising contacting a library of two or more candidate agents with a minicell comprising said protein or a polypeptide derived from said protein, assaying the effect of candidate

agents on the activity of said protein, and identifying agents that effect the activity of said protein.

374. The method of claim 373, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

375. The method of claim 373, wherein said protein or said polypeptide derived from said protein is displayed on the surface of said minicell.

376. The method of claim 373, wherein said protein is a membrane protein.

377. The method of claim 376, wherein said membrane protein is selected from the group consisting of a receptor, a channel protein and an enzyme.

378. The method of claim 373, wherein said activity of a protein is a binding activity or an enzymatic activity.

379. The method of claim 373, wherein said library of compounds is a protein library.

380. The method of claim 379, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

381. The method of claim 373, wherein said library of compounds is a library of aptamers.

382. The method of claim 373, wherein said library of compounds is a library of small molecules.

383. A method of identifying an agent that effects the activity of a protein domain containing a library of two or more candidate agents with a minicell displaying a membrane fusion protein, said fusion protein comprising a first polypeptide, said first polypeptide comprising at least one transmembrane domain or at least one membrane anchoring domain, and a second polypeptide, wherein said second polypeptide comprises said protein domain.

384. A method of identifying undesirable side-effects of a biologically active compound that occur as a result of binding of said compound to a protein, wherein binding a compound to said protein is known to result in undesirable side effects, comprising contacting a minicell that comprises said protein to said biologically active compound.

385. The method of claim 384, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

386. The method of claim 384, further comprising characterizing the binding of said biologically active compound to said protein.

387. The method of claim 384, further comprising characterizing the effect of said biologically active compound on the activity of said protein.

388. A method for identifying an agent that effects the interaction of a first signaling protein with a second signaling protein, comprising

(a) contacting a library of compounds with a minicell, wherein said minicell comprises:

(i) a first protein comprising said first signaling protein and a first trans-acting regulatory domain;

(ii) a second protein comprising said second signaling protein and a second trans-acting regulatory domain; and

(iii) a reporter gene, the expression of which is modulated by the interaction between said first trans-acting regulatory domain and said second trans-acting regulatory domain; and

(b) detecting the gene product of said reporter gene.

389. The method of claim 388, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

390. The method of claim 388, wherein said trans-acting regulatory domains are transactivation domains.

391. The method of claim 388, wherein said trans-acting regulatory domains are transrepression domains.

392. The method of claim 388, wherein said reporter gene is induced by the interaction of said first trans-acting regulatory domain and said second trans-acting regulatory domain.

393. The method of claim 388, wherein said agent that effects the interaction of said first signaling protein with said second signaling protein is an agent that causes or promotes said interaction.

394. The method of claim 388, wherein said reporter gene is repressed by the interaction of said first trans-acting regulatory domain and said second trans-acting regulatory domain.

395. The method of claim 394, wherein said agent that effects the interaction of said first signaling protein with said second signaling protein is an agent that inhibits or blocks said interaction.

396. The method of claim 388, wherein said first signaling protein is a GPCR.

397. The method of claim 396, wherein said GPCR is an Edg receptor or a ScAMPER.

398. The method of claim 396, wherein said second signalling protein is a G-protein..

399. The method of claim 398, wherein said G-protein is selected from the group consisting of G-alpha-i, G-alpha-s, G-alpha-q, G-alpha-12/13 and Go.

400. The method of claim 388, wherein said library of compounds is a protein library.

401. The method of claim 400, wherein said protein library is selected from the group consisting of a phage display library, a phagemid display library, and a ribosomal display library.

402. The method of claim 388, wherein said library of compounds is a library of aptamers.

403. The method of claim 388, wherein said library of compounds is a library of small molecules.

404. A method for identifying an agent that effects the interaction of a first signaling protein with a second signaling protein, comprising contacting a library of two or more candidate agents with a minicell, wherein said minicell comprises:

(a) a first fusion protein comprising said first signaling protein and a first detectable domain; and

(b) a second fusion protein comprising said second signaling protein and a second detectable domain,

wherein a signal is generated when said first and second signaling proteins are in close proximity to each other, and detecting said signal.

405. The method of claim 404, wherein said signal is fluorescence.

406. The method of claim 404, wherein said first detectable domain and said second detectable domain are fluorescent and said signal is generated by FRET.

407. The method of claim 406, wherein said first and second detectable domains are independently selected from the group consisting of a green fluorescent protein, a blue-shifted green fluorescent protein, a cyan-shifted green fluorescent protein; a red-shifted green fluorescent protein; a yellow-shifted green fluorescent protein, and a red fluorescent protein, wherein said first fluorescent domain and said second fluorescent domain are not identical.

408. A method of bioremediation, said method comprising contacting a composition that comprises an undesirable substance with a minicell, wherein said minicell alters the chemical structure and/or binds said undesirable substance.

409. A method of bioremediation, said method comprising contacting a composition that comprises an undesirable substance with a minicell, wherein said mincell comprises an agent that alters the chemical structure of said undesirable substance.

410. The method of claim 409, wherein said agent that alters the chemical structure of said undesirable substance is an inorganic catalyst.

411. The method of claim 409, wherein said agent that alters the chemical structure of said undesirable substance is an enzyme.

412. The method of claim 411, wherein said enzyme is a soluble protein contained within said minicell.

413. The method of claim 412, wherein said soluble protein is selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

414. The method of claim 411, wherein said enzyme is a secreted protein.

415. The method of claim 414, wherein said secreted protein is selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

416. The method of claim 411, wherein said enzyme is a membrane protein.

417. The method of claim 416, wherein said membrane enzyme is selected from the group consisting of a cytochrome P450, an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

418. The method of claim 409, wherein said agent that alters the chemical structure of said undesirable substance is a fusion protein comprising a first polypeptide that comprises a transmembrane domain or at least one membrane-anchoring domain, and a second polypeptide, wherein said second polypeptide is an enzyme moiety.

419. The method of claim 418, wherein said second polypeptide is a polypeptide derived from a protein selected from the group consisting of an oxidoreductase, a transferase, a hydrolase, a lyase, an isomerase, a ligase and a synthetase.

420. A method of bioremediation, said method comprising contacting a composition that comprises an undesirable substance with a minicell, wherein said mincell comprises an agent that binds an undesirable substance.

421. The method of claim 420, wherein said undesirable substance binds to and is internalized by said minicell or is otherwise inactivated by selective absorption.

422. The method of claim 420, wherein said agent that binds said undesirable substance is a secreted soluble protein.

423. The method of claim 422, wherein said secreted protein is a transport accessory protein.

424. The method of claim 420, wherein said agent that binds said undesirable substance is a membrane protein.

425. The method of claim 420, wherein said undesirable substance is selected from the group consisting of a toxin, a pollutant and a pathogen.

426. The method of claim 420, wherein said agent that binds said undesirable substance is a fusion protein comprising a first polypeptide that comprises a transmembrane domain or at least one membrane-anchoring domain, and a second polypeptide, wherein said second polypeptide is a binding moiety.

427. The method of claim 426, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, the active site of a non-enzymatically active mutant enzyme, a single-chain antibody and an aptamer.

428. A minicell-producing parent cell, wherein said parent cell comprises one or more of the following:

(a) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or

repression of said gene regulates the copy number of an episomal expression construct;

(b) a mutation in an endogenous gene, wherein said mutation regulates the copy number of an episomal expression construct;

(c) an expression element that comprises a gene operably linked to expression sequences that are inducible and/or repressible, wherein induction or repression of said gene causes or enhances the production of minicells; and

(d) a mutation in an endogenous gene, wherein said mutation causes or enhances minicell production.

429. The minicell-producing parent cell of claim 428, further comprising an episomal expression construct.

430. The minicell-producing parent cell of claim 428, further comprising a chromosomal expression construct.

431. The minicell-producing parent cell of claim 429, wherein said expression sequences of said expression construct are inducible and/or repressible.

432. The minicell-producing parent cell of claim 428, wherein said minicell-producing parent cell comprises a biologically active compound.

433. The minicell of claim 428 wherein said gene that causes or enhances the production of minicells has a gene product that is involved in or regulates DNA replication, cellular division, cellular partitioning, septation, transcription, translation, or protein folding.

434. A minicell-producing parent cell, wherein said parent cell comprises an expression construct, wherein said expression construct comprises expression sequences operably linked to an ORF that encodes a protein, and a regulatory expression element, wherein said regulatory expression element comprises expression sequences operably linked to a regulatory gene that encodes a factor that regulates the expression of said ORF.

435. The minicell-producing parent cell of claim 434, wherein said expression sequences of said expression construct are inducible and/or repressible.

436. The minicell-producing parent cell of claim 434, wherein said expression sequences of said regulatory expression construct are inducible and/or repressible.

437. The minicell-producing parent cell of claim 434, wherein one or more of said expression element or said regulatory expression element is located on a chromosome of said parent cell.

438. The minicell-producing parent cell of claim 434, wherein one or more of said expression element or said regulatory expression element is located on an episomal expression construct.

439. The minicell-producing parent cell of claim 438, wherein both of said expression element and said regulatory expression element are located on an episomal expression construct, and one or both of said expression element and said regulatory expression element segregates into minicells produced from said parent cell.

440. The minicell-producing parent cell of claim 434, wherein said minicell-producing parent cell comprises a biologically active compound.

441. The minicell-producing parent cell of claim 440, wherein said biologically active compound segregates into minicells produced from said parent cell.

442. The minicell-producing parent cell of claim 434, wherein said ORF encodes a membrane protein or a soluble protein.

443. The minicell-producing parent cell of claim 434, wherein said protein comprises secretion sequences.

444. The minicell-producing parent cell of claim 434, wherein the gene product of said gene regulates the expression of said ORF.

445. The minicell-producing parent cell of claim 444, wherein said gene product is a transcription factor.

446. The minicell-producing parent cell of claim 440, wherein said gene product is a RNA polymerase.

447. The minicell-producing parent cell of claim 446, wherein said parent cell is MC-T7.

448. A minicell comprising a biologically active compound, wherein said minicell displays a binding moiety, wherein said minicell selectively absorbs and/or internalizes an undesirable compound, and said minicell is a poroplast, spheroplast or protoplast.

449. The minicell of claim 448, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a receptor and an active site of a non-catalytic derivative of an enzyme.

450. The minicell of claim 458, wherein said binding moiety is a single-chain antibody.

451. The minicell of claim 458, wherein said binding moiety is directed to a ligand selected from the group consisting of an epitope displayed on a pathogen, an epitope displayed on an infected cell and an epitope displayed on a hyperproliferative cell.

452. The minicell of claim 458, wherein said biologically active compound is selected from the group consisting of a radioisotope, a polypeptide, a nucleic acid and a small molecule.

453. The minicell of claim 448, wherein a ligand binds to and is internalized by said minicell or is otherwise inactivated by selective absorption.

454. A pharmaceutical composition comprising the minicell of claim 448.

455. A method of reducing the free concentration of a substance in a composition, wherein said substance displays a ligand specifically recognized by a binding moiety, comprising contacting said composition with a minicell that displays said binding moiety, wherein said binding moiety binds said substance, thereby reducing the free concentration of said substance in said composition.

456. The method of claim 455, wherein said minicell is selected from the group consisting of a eubacterial minicell, a poroplast, a spheroplast and a protoplast.

457. The method of claim 455, wherein said substance is selected from the group consisting of a nucleic acid, a lipid, a polypeptide, a radioactive compound, an ion and a small molecule.

458. The method of claim 455, wherein said binding moiety is selected from the group consisting of an antibody, an antibody derivative, a channel protein and a receptor.

459. The method of claim 455, wherein said composition is present in an environment.

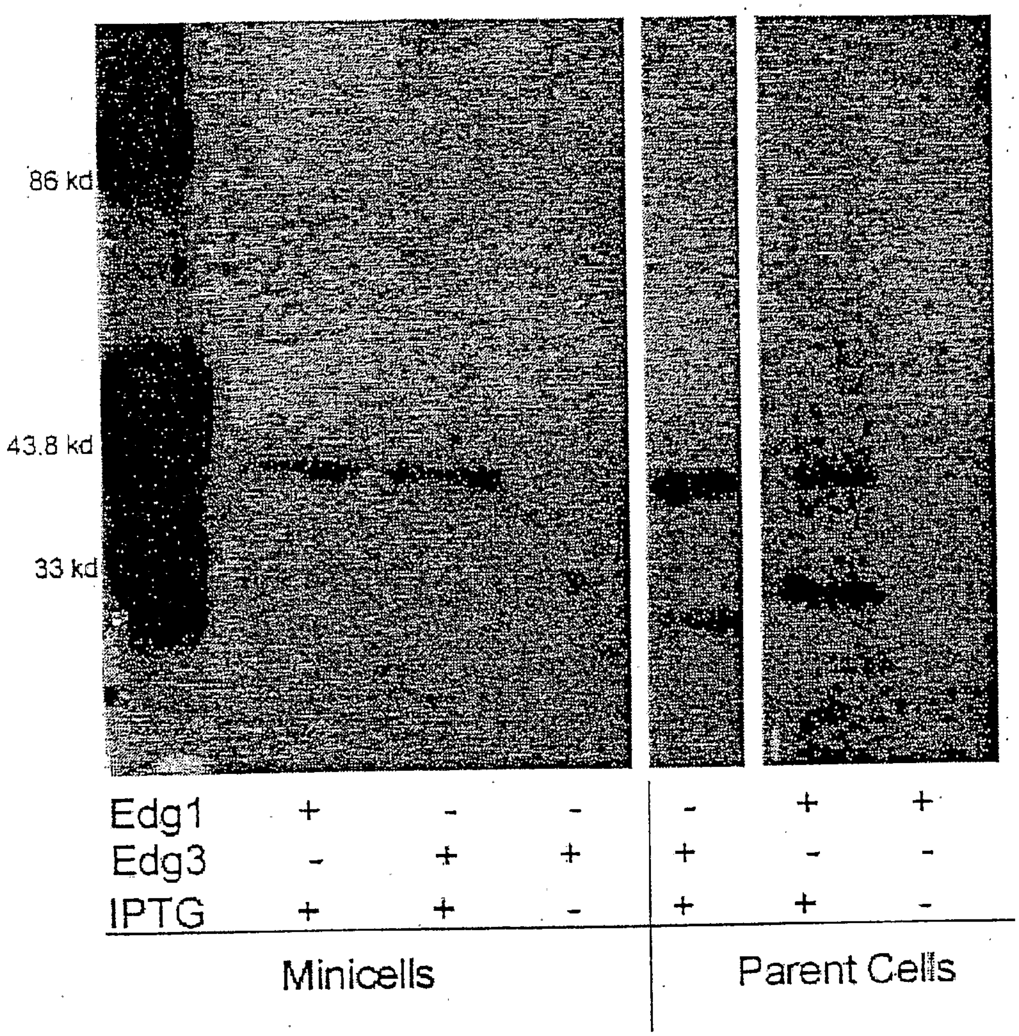
460. The method of claim 459, wherein said environment is water, air or soil.

461. The method of claim455, wherein said composition is a biological sample from an organism.

462. The method of claim 461, wherein said biological sample is selected from the group consisting of blood, serum, plasma, urine, saliva, a biopsy sample, feces, tissue and a skin patch.

463. The method of claim 461, wherein said substance binds to and is internalized by said minicell or is otherwise inactivated by selective absorption.

464. The method of claim 463, wherein said biological sample is returned to said organism after being contacting to said minicell.


86 kd
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33 kd
Edg1 + - - - + +
Edg3 - + + + - -
IPTG + + - + + -
Minicells
Parent Cells

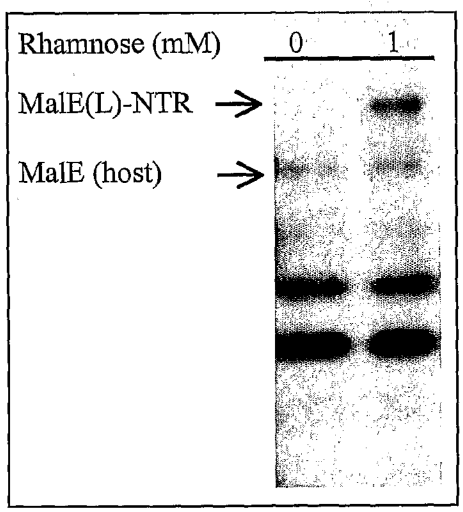

Figure 2

SEQUENCE LISTING

SEQ ID NO 1

pMPX-23 (complete *ftsZ* cloned into pMPX-18 using PCR-introduced PstI and XbaI)

```
                                Shine-Delgarno       PstI
1621     CCATACCCGTTTTTTTGGGCTAGCAGGAGGAATTCACCCTGCAGATGTTTGAACCAATGG
1                                                    M  F  E  P  M

1681     AACTTACCAATGACGCGGTGATTAAAGTCATCGGCGTCGGCGGCGGCGGCGGTAATGCTG
6        E  L  T  N  D  A  V  I  K  V  I  G  V  G  G  G  G  G  N  A

1741     TTGAACACATGGTGCGCGAGCGCATTGAAGGTGTTGAATTCTTCGCGGTAAATACCGATG
26       V  E  H  M  V  R  E  R  I  E  G  V  E  F  F  A  V  N  T  D

1801     CACAAGCGCTGCGTAAAACAGCGGTTGGACAGACGATTCAAATCGGTAGCGGTATCACCA
46       A  Q  A  L  R  K  T  A  V  G  Q  T  I  Q  I  G  S  G  I  T

1861     AAGGACTGGGCGCTGGCGCTAATCCAGAAGTTGGCCGCAATGCGGCTGATGAGGATCGCG
66       K  G  L  G  A  G  A  N  P  E  V  G  R  N  A  A  D  E  D  R

1921     ATGCATTGCGTGCGGCGCTGGAAGGTGCAGACATGGTCTTTATTGCTGCGGGTATGGGTG
86       D  A  L  R  A  A  L  E  G  A  D  M  V  F  I  A  A  G  M  G

1981     GTGGTACCGGTACAGGTGCAGCACCAGTCGTCGCTGAAGTGGCAAAAGATTTGGGTATCC
106      G  G  T  G  T  G  A  A  P  V  V  A  E  V  A  K  D  L  G  I

2041     TGACCGTTGCTGTCGTCACTAAGCCTTTCAACTTTGAAGGCAAGAAGCGTATGGCATTCG
126      L  T  V  A  V  V  T  K  P  F  N  F  E  G  K  K  R  M  A  F

2101     CGGAGCAGGGGATCACTGAACTGTCCAAGCATGTGGACTCTCTGATCACTATCCCGAACG
146      A  E  Q  G  I  T  E  L  S  K  H  V  D  S  L  I  T  I  P  N

2161     ACAAACTGCTGAAAGTTCTGGGCCGCGGTATCTCCCTGCTGGATGCGTTTGGCGCAGCGA
166      D  K  L  L  K  V  L  G  R  G  I  S  L  L  D  A  F  G  A  A

2221     ACGATGTACTGAAAGGCGCTGTGCAAGGTATCGCTGAACTGATTACTCGTCCGGGTTTGA
186      N  D  V  L  K  G  A  V  Q  G  I  A  E  L  I  T  R  P  G  L

2281     TGAACGTGGACTTTGCAGACGTACGCACCGTAATGTCTGAGATGGGCTACGCAATGATGG
206      M  N  V  D  F  A  D  V  R  T  V  M  S  E  M  G  Y  A  M  M

2341     GTTCTGGCGTGGCGAGCGGTGAAGACCGTGCGGAAGAAGCTGCTGAAATGGCTATCTCTT
226      G  S  G  V  A  S  G  E  D  R  A  E  E  A  A  E  M  A  I  S

2401     CTCCGCTGCTGGAAGATATCGACCTGTCTGGCGCGCGCGGCGTGCTGGTTAACATCACGG
246      S  P  L  L  E  D  I  D  L  S  G  A  R  G  V  L  V  N  I  T

2461     CGGGCTTCGACCTGCGTCTGGATGAGTTCGAAACGGTAGGTAACACCATCCGTGCATTTG
266      A  G  F  D  L  R  L  D  E  F  E  T  V  G  N  T  I  R  A  F

2521     CTTCCGACAACGCGACTGTGGTTATCGGTACTTCTCTTGACCCGGATATGAATGACGAGC
286      A  S  D  N  A  T  V  V  I  G  T  S  L  D  P  D  M  N  D  E

2581     TGCGCGTAACCGTTGTTGCGACAGGTATCGGCATGGACAAACGTCCTGAAATCACTCTGG
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306     L  R  V  T  V  V  A  T  G  I  G  M  D  K  R  P  E  I  T  L

2641    TGACCAATAAGCAGGTTCAGCAGCCAGTGATGGATCGCTACCAGCAGCATGGGATGGCTC
326     V  T  N  K  Q  V  Q  Q  P  V  M  D  R  Y  Q  Q  H  G  M  A

2701    CGCTGACCCAGGAGCAGAAGCCGGTTGCTAAAGTCGTGAATGACAATGCGCCGCAAACTG
346     P  L  T  Q  E  Q  K  P  V  A  K  V  V  N  D  N  A  P  Q  T

2761    CGAAAGAGCCGGATTATCTGGATATCCCAGCATTCCTGCGTAAGCAAGCTGATTAATAAT
366     A  K  E  P  D  Y  L  D  I  P  A  F  L  R  K  Q  A  D

        XbaI
2821    CTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCTGTG
```

Sequence contains full-length *ftsZ* PCR amplified from *E. coli* MG1655 using oligos containing PstI and XbaI restriction sites.

SEQ ID NO 2

pMPX-47 (complete *ftsZ* cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                                 Shine-Delgarno    PstI
2401    GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
                                                                    M

2461    GTTTGAACCAATGGAACTTACCAATGACGCGGTGATTAAAGTCATCGGCGTCGGCGGCGG
2        F  E  P  M  E  L  T  N  D  A  V  I  K  V  I  G  V  G  G  G

2521    CGGCGGTAATGCTGTTGAACACATGGTGCGCGAGCGCATTGAAGGTGTTGAATTCTTCGC
22       G  G  N  A  V  E  H  M  V  R  E  R  I  E  G  V  E  F  F  A

2581    GGTAAATACCGATGCACAAGCGCTGCGTAAAACAGCGGTTGGACAGACGATTCAAATCGG
42       V  N  T  D  A  Q  A  L  R  K  T  A  V  G  Q  T  I  Q  I  G

2641    TAGCGGTATCACCAAAGGACTGGGCGCTGGCGCTAATCCAGAAGTTGGCCGCAATGCGGC
62       S  G  I  T  K  G  L  G  A  G  A  N  P  E  V  G  R  N  A  A

2701    TGATGAGGATCGCGATGCATTGCGTGCGGCGCTGGAAGGTGCAGACATGGTCTTTATTGC
82       D  E  D  R  D  A  L  R  A  A  L  E  G  A  D  M  V  F  I  A

2761    TGCGGGTATGGGTGGTGGTACCGGTACAGGTGCAGCACCAGTCGTCGCTGAAGTGGCAAA
102      A  G  M  G  G  G  T  G  T  G  A  A  P  V  V  A  E  V  A  K

2821    AGATTTGGGTATCCTGACCGTTGCTGTCGTCACTAAGCCTTTCAACTTTGAAGGCAAGAA
122      D  L  G  I  L  T  V  A  V  V  T  K  P  F  N  F  E  G  K  K

2881    GCGTATGGCATTCGCGGAGCAGGGGATCACTGAACTGTCCAAGCATGTGGACTCTCTGAT
142      R  M  A  F  A  E  Q  G  I  T  E  L  S  K  H  V  D  S  L  I

2941    CACTATCCCGAACGACAAACTGCTGAAAGTTCTGGGCCGCGGTATCTCCCTGCTGGATGC
162      T  I  P  N  D  K  L  L  K  V  L  G  R  G  I  S  L  L  D  A

3001    GTTTGGCGCAGCGAACGATGTACTGAAAGGCGCTGTGCAAGGTATCGCTGAACTGATTAC
182      F  G  A  A  N  D  V  L  K  G  A  V  Q  G  I  A  E  L  I  T

3061    TCGTCCGGGTTTGATGAACGTGGACTTTGCAGACGTACGCACCGTAATGTCTGAGATGGG
```

```
202        R  P  G  L  M  N  V  D  F  A  D  V  R  T  V  M  S  E  M  G

3121     CTACGCAATGATGGGTTCTGGCGTGGCGAGCGGTGAAGACCGTGCGGAAGAAGCTGCTGA
222        Y  A  M  M  G  S  G  V  A  S  G  E  D  R  A  E  E  A  A  E

3181     AATGGCTATCTCTTCTCCGCTGCTGGAAGATATCGACCTGTCTGGCGCGCGCGGCGTGCT
242        M  A  I  S  S  P  L  L  E  D  I  D  L  S  G  A  R  G  V  L

3241     GGTTAACATCACGGCGGGCTTCGACCTGCGTCTGGATGAGTTCGAAACGGTAGGTAACAC
262        V  N  I  T  A  G  F  D  L  R  L  D  E  F  E  T  V  G  N  T

3301     CATCCGTGCATTTGCTTCCGACAACGCGACTGTGGTTATCGGTACTTCTCTTGACCCGGA
282        I  R  A  F  A  S  D  N  A  T  V  V  I  G  T  S  L  D  P  D

3361     TATGAATGACGAGCTGCGCGTAACCGTTGTTGCGACAGGTATCGGCATGGACAAACGTCC
302        M  N  D  E  L  R  V  T  V  V  A  T  G  I  G  M  D  K  R  P

3421     TGAAATCACTCTGGTGACCAATAAGCAGGTTCAGCAGCCAGTGATGGATCGCTACCAGCA
322        E  I  T  L  V  T  N  K  Q  V  Q  Q  P  V  M  D  R  Y  Q  Q

3481     GCATGGGATGGCTCCGCTGACCCAGGAGCAGAAGCCGGTTGCTAAAGTCGTGAATGACAA
342        H  G  M  A  P  L  T  Q  E  Q  K  P  V  A  K  V  V  N  D  N

3541     TGCGCCGCAAACTGCGAAAGAGCCGGATTATCTGGATATCCCAGCATTCCTGCGTAAGCA
362        A  P  Q  T  A  K  E  P  D  Y  L  D  I  P  A  F  L  R  K  Q

                         XbaI
3601     AGCTGATTAATAATCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCAT
382        A  D
```

Sequence contains full-length *ftsZ* PCR amplified from *E. coli* MG1655 using oligos containing PstI and XbaI restriction sites.

SEQ ID NO 3

araC::Para::*ftsZ* inserted by RED recombination into *E. coli* MG1655 *intD*

```
        intD homology for recombination                       Stop araC
181     AAGCCTGCAT TGCGGCGCTT CAGTCTCCGC TGCATACTGT CCCGTTACCA
ATTATGACAA

241     CTTGACGGCT ACATCATTCA CTTTTTCTTC ACAACCGGCA CGGAACTCGC
TCGGGCTGGC
301     CCCGGTGCAT TTTTTAAATA CCCGCGAGAA ATAGAGTTGA TCGTCAAAAC
CAACATTGCG
361     ACCGACGGTG GCGATAGGCA TCCGGGTGGT GCTCAAAAGC AGCTTCGCCT
GGCTGATACG
421     TTGGTCCTCG CGCCAGCTTA AGACGCTAAT CCCTAACTGC TGGCGGAAAA
GATGTGACAG
481     ACGCGACGGC GACAAGCAAA CATGCTGTGC GACGCTGGCG ATATCAAAAT
TGCTGTCTGC
541     CAGGTGATCG CTGATGTACT GACAAGCCTC GCGTACCCGA TTATCCATCG
GTGGATGGAG
```

601 CGACTCGTTA ATCGCTTCCA TGCGCCGCAG TAACAATTGC TCAAGCAGAT TTATCGCCAG
661 CAGCTCCGAA TAGCGCCCTT CCCCTTGCCC GGCGTTAATG ATTTGCCCAA ACAGGTCGCT
721 GAAATGCGGC TGGTGCGCTT CATCCGGGCG AAAGAACCCC GTATTGGCAA ATATTGACGG
781 CCAGTTAAGC CATTCATGCC AGTAGGCGCG CGGACGAAAG TAAACCCACT GGTGATACCA
841 TTCGCGAGCC TCCGGATGAC GACCGTAGTG ATGAATCTCT CCTGGCGGGA ACAGCAAAAT
901 ATCACCCGGT CGGCAAACAA ATTCTCGTCC CTGATTTTTC ACCACCCCCT GACCGCGAAT
961 GGTGAGATTG AGAATATAAC CTTTCATTCC CAGCGGTCGG TCGATAAAAA AATCGAGATA
1021 ACCGTTGGCC TCAATCGGCG TTAAACCCGC CACCAGATGG GCATTAAACG AGTATCCCGG
1081 CAGCAGGGGA TCATTTTGCG CTTCAGCCAT ACTTTTCATA CTCCCGCCAT TCAGAGAAGA

Start *araC*
1141 AACCAATTGT CCATATTG**CA T**CAGACATTG *CCGTCACTGC GTCTTTTACT GGCTCTTCTC*
←

1201 *GCTAACCAAA CCGGTAACCC CGCTTATTAA AAGCATTCTG TAACAAAGCG GGACCAAAGC*
1261 *CATGACAAAA ACGCGTAACA AAAGTGTCTA TAATCACGGC AGAAAAGTCC ACATTGATTA*
1321 *TTTGCACGGC GTCACACTTT GCTATGCCAT AGCATTTTTA TCCATAAGAT TAGCGGATCC*
1381 *TACCTGACGC TTTTTATCGC AACTCTCTAC TGTTTCTCCA TACCCGTTTT TTTGGGCTAG*

Shine-Delgarno Start *ftsZ*
1441 *CAGGAGGAAT TCACCCTGCA* G**ATG**TTTGAA CCAATGGAAC TTACCAATGA CGCGGTGATT
→

1501 AAAGTCATCG GCGTCGGCGG CGGCGGCGGT AATGCTGTTG AACACATGGT GCGCGAGCGC
1561 ATTGAAGGTG TTGAATTCTT CGCGGTAAAT ACCGATGCAC AAGCGCTGCG TAAAACAGCG
1621 GTTGGACAGA CGATTCAAAT CGGTAGCGGT ATCACCAAAG GACTGGGCGC TGGCGCTAAT
1681 CCAGAAGTTG GCCGCAATGC GGCTGATGAG GATCGCGATG CATTGCGTGC GGCGCTGGAA
1741 GGTGCAGACA TGGTCTTTAT TGCTGCGGGT ATGGGTGGTG GTACCGGTAC AGGTGCAGCA
1801 CCAGTCGTCG CTGAAGTGGC AAAAGATTTG GGTATCCTGA CCGTTGCTGT CGTCACTAAG
1861 CCTTTCAACT TTGAAGGCAA GAAGCGTATG GCATTCGCGG AGCAGGGGAT CACTGAACTG
1921 TCCAAGCATG TGGACTCTCT GATCACTATC CCGAACGACA AACTGCTGAA AGTTCTGGGC
1981 CGCGGTATCT CCCTGCTGGA TGCGTTTGGC GCAGCGAACG ATGTACTGAA AGGCGCTGTG
2041 CAAGGTATCG CTGAACTGAT TACTCGTCCG GGTTTGATGA ACGTGGACTT TGCAGACGTA
2101 CGCACCGTAA TGTCTGAGAT GGGCTACGCA ATGATGGGTT CTGGCGTGGC GAGCGGTGAA

2161 GACCGTGCGG AAGAAGCTGC TGAAATGGCT ATCTCTTCTC CGCTGCTGGA AGATATCGAC
2221 CTGTCTGGCG CGCGCGGCGT GCTGGTTAAC ATCACGGCGG GCTTCGACCT GCGTCTGGAT
2281 GAGTTCGAAA CGGTAGGTAA CACCATCCGT GCATTTGCTT CCGACAACGC GACTGTGGTT
2341 ATCGGTACTT CTCTTGACCC GGATATGAAT GACGAGCTGC GCGTAACCGT TGTTGCGACA
2401 GGTATCGGCA TGGACAAACG TCCTGAAATC ACTCTGGTGA CCAATAAGCA GGTTCAGCAG
2461 CCAGTGATGG ATCGCTACCA GCAGCATGGG ATGGCTCCGC TGACCCAGGA GCAGAAGCCG
2521 GTTGCTAAAG TCGTGAATGA CAATGCGCCG CAAACTGCGA AAGAGCCGGA TTATCTGGAT

Stop *ftsZ*
2581 ATCCCAGCAT TCCTGCGTAA GCAAGCTGAT TAATAATCTA GAGGCGTTAC CAATTATGAC

FRT scar *intD* **homology**
2641 AACTTGACGG **GAAGTTCCTA TACTTTCTAG AGAATAGGAA CTTC*CC AAAG CCAGTATCAA***

for recombination
3721 ***CTCAGACAAA GGCAAAGCAT CTTG***

Bold, italicized represents homology between the PCR product shown below and *intD*.

araC::Para::*ftsZ*::FRT::*kan*::Frt

Following RED recombination into *intD*, the kanamycin cassette was removed with *flp* recombinase resulting in a single FRT scar as depicted above. Bold alone represents FRT scar after the *flp* reaction.

SEQ ID NO 4

rhaRS::Prha::*ftsZ* inserted by RED recombination into *E. coli* MG1655 *intD*

***intD* homology for recombination** Stop *rhaR*
181 ***AAGCCTGCAT TGCGGCGCTT CAGTCTCCGC TGCATACTGT CC***TTAATCTT TCTGCGAATT
241 GAGATGACGC CACTGGCTGG GCGTCATCCC GGTTTCCCGG GTAAACACCA CCGAAAAATA
301 GTTACTATCT TCAAAGCCAC ATTCGGTCGA AATATCACTG ATTAACAGGC GGCTATGCTG
361 GAGAAGATAT TGCGCATGAC ACACTCTGAC CTGTCGCAGA TATTGATTGA TGGTCATTCC
421 AGTCTGCTGG CGAAATTGCT GACGCAAAAC GCGCTCACTG CACGATGCCT CATCACAAAA
481 TTTATCCAGC GCAAAGGGAC TTTTCAGGCT AGCCGCCAGC CGGGTAATCA GCTTATCCAG

541 CAACGTTTCG CTGGATGTTG GCGGCAACGA ATCACTGGTG TAACGATGGC GATTCAGCAA
601 CATCACCAAC TGCCCGAACA GCAACTCAGC CATTTCGTTA GCAAACGGCA CATGCTGACT
661 ACTTTCATGC TCAAGCTGAC CGATAACCTG CCGCGCCTGC GCCATCCCCA TGCTACCTAA
721 GCGCCAGTGT GGTTGCCCTG CGCTGGCGTT AAATCCCGGA ATCGCCCCCT GCCAGTCAAG
781 ATTCAGCTTC AGACGCTCCG GGCAATAAAT AATATTCTGC AAAACCAGAT CGTTAACGGA
841 AGCGTAGGAG TGTTTATCGT CAGCATGAAT GTAAAAGAGA TCGCCACGGG TAATGCGATA
901 AGGGCGATCG TTGAGTACAT GCAGGCCATT ACCGCGCCAG ACAATCACCA GCTCACAAAA
961 ATCATGTGTA TGTTCAGCAA AGACATCTTG CGGATAACGG TCAGCCACAG CGACTGCCTG
1021 CTGGTCGCTG GCAAAAAAAT CATCTTTGAG AAGTTTTAAC TGATGCGCCA CCGTGGCTAC
1081 CTCGGCCAGA GAACGAAGTT GATTATTCGC AATATGGCGT ACAAATACGT TGAGAAGATT

Stop *rhaS* Start *rhaR*
1141 CGCG**TTA**TTG CAGAAAGC**CA T**CCCGTCCCT GGCGAATATC ACGCGGTGAC CAGTTAAACT

←

1201 CTCGGCGAAA AAGCGTCGAA AAGTGGTTAC TGTCGCTGAA TCCACAGCGA TAGGCGATGT
1261 CAGTAACGCT GGCCTCGCTG TGGCGTAGCA GATGTCGGGC TTTCATCAGT CGCAGGCGGT
1321 TCAGGTATCG CTGAGGCGTC AGTCCCGTTT GCTGCTTAAG CTGCCGATGT AGCGTACGCA
1381 GTGAAAGAGA AAATTGATCC GCCACGGCAT CCCAATTCAC CTCATCGGCA AAATGGTCCT
1441 CCAGCCAGGC CAGAAGCAAG TTGAGACGTG ATGCGCTGTT TTCCAGGTTC TCCTGCAAAC
1501 TGCTTTTACG CAGCAAGAGC AGTAATTGCA TAAACAAGAT CTCGCGACTG GCGGTCGAGG
1561 GTAAATCATT TTCCCCTTCC TGCTGTTCCA TCTGTGCAAC CAGCTGTCGC ACCTGCTGCA
1621 ATACGCTGTG GTTAACGCGC CAGTGAGACG GATACTGCCC ATCCAGCTCT TGTGGCAGCA
1681 ACTGATTCAG CCCGGCGAGA AACTGAAATC GATCCGGCGA GCGATACAGC ACATTGGTCA
1741 GACACAGATT ATCGGTATGT TCATACAGAT GCCGATCATG ATCGCGTACG AAACAGACCG
1801 TGCCACCGGT GATGGTATAG GGCTGCCCAT TAAACACATG AATACCCGTG CCATGTTCGA
1861 CAATCACAAT TTCATGAAAA TCATGATGAT GTTCAGGAAA ATCCGCCTGC GGGAGCCGGG

Start *rhaS*
1921 GTTCTATCGC CACGGACGCG TTACCAGACG GAAAAAAATC CACACTATGT AATACGGT<u>CA</u>

←

1981 <u>T</u>ACTGGCCTC CTGATGTCGT CAACACGGCG AAATAGTAAT CACGAGGTCA GGTTCTTACC

2041 TTAAATTTTC GACGGAAAAC CACGTAAAAA ACGTCGATTT TTCAAGATAC AGCGTGAATT
2101 TTCAGGAAAT GCGGTGAGCA TCACATCACC ACAATTCAGC AAATTGTGAA CATCATCACG
2161 TTCATCTTTC CCTGGTTGCC AATGGCCCAT TTTCCTGTCA GTAACGAGAA GGTCGCGAAT

Shine-Delgarno Start *ftsZ*
2221 TCAGGCGCTT TTTAGACTGG TCGTAATGAA ATTCAGCAGG ATCACAT**ATG** TTTGAACCAA

→

2581 TGGAACTTAC CAATGACGCG GTGATTAAAG TCATCGGCGT CGGCGGCGGC GGCGGTAATG
2641 CTGTTGAACA CATGGTGCGC GAGCGCATTG AAGGTGTTGA ATTCTTCGCG GTAAATACCG
2701 ATGCACAAGC GCTGCGTAAA ACAGCGGTTG GACAGACGAT TCAAATCGGT AGCGGTATCA
2761 CCAAAGGACT GGGCGCTGGC GCTAATCCAG AAGTTGGCCG CAATGCGGCT GATGAGGATC
2821 GCGATGCATT GCGTGCGGCG CTGGAAGGTG CAGACATGGT CTTTATTGCT GCGGGTATGG
2881 GTGGTGGTAC CGGTACAGGT GCAGCACCAG TCGTCGCTGA AGTGGCAAAA GATTTGGGTA
2941 TCCTGACCGT TGCTGTCGTC ACTAAGCCTT TCAACTTTGA AGGCAAGAAG CGTATGGCAT
3001 TCGCGGAGCA GGGGATCACT GAACTGTCCA AGCATGTGGA CTCTCTGATC ACTATCCCGA
3061 ACGACAAACT GCTGAAAGTT CTGGGCCGCG GTATCTCCCT GCTGGATGCG TTTGGCGCAG
3121 CGAACGATGT ACTGAAAGGC GCTGTGCAAG GTATCGCTGA ACTGATTACT CGTCCGGGTT
3181 TGATGAACGT GGACTTTGCA GACGTACGCA CCGTAATGTC TGAGATGGGC TACGCAATGA
3241 TGGGTTCTGG CGTGGCGAGC GGTGAAGACC GTGCGGAAGA AGCTGCTGAA ATGGCTATCT
3301 CTTCTCCGCT GCTGGAAGAT ATCGACCTGT CTGGCGCGCG CGGCGTGCTG GTTAACATCA
3361 CGGCGGGCTT CGACCTGCGT CTGGATGAGT TCGAAACGGT AGGTAACACC ATCCGTGCAT
3421 TTGCTTCCGA CAACGCGACT GTGGTTATCG GTACTTCTCT TGACCCGGAT ATGAATGACG
3481 AGCTGCGCGT AACCGTTGTT GCGACAGGTA TCGGCATGGA CAAACGTCCT GAAATCACTC
3541 TGGTGACCAA TAAGCAGGTT CAGCAGCCAG TGATGGATCG CTACCAGCAG CATGGGATGG
3601 CTCCGCTGAC CCAGGAGCAG AAGCCGGTTG CTAAAGTCGT GAATGACAAT GCGCCGCAAA

Stop *ftsZ*
3661 CTGCGAAAGA GCCGGATTAT CTGGATATCC CAGCATTCCT GCGTAAGCAA GCTGAT**TAA**T

FRT scar
3721 AATCTAGAGG CGTTACCAAT TATGACAACT TGACGG**GAAG TTCCTATACT TTCTAGAGAA**

intD homology for recombination

3781 **TAGGAACTTC *CCAAAGCCAG TATCAACTCA GACAAAGGCA AAGCATCTTG***

Bold, italicized represents homology between the PCR product shown below and *intD*.

rhaRS::Prha::*ftsZ*::FRT::*kan*::Frt

Following RED recombination into *intD*, the kanamycin cassette was removed with *flp* recombinase resulting in a single FRT scar as depicted above. Bold alone represents FRT scar after the *flp* reaction.

SEQ ID NO 5

lacI::Ptac::*ftsZ* inserted by RED recombination into *E. coli* MG1655 *intD*

***intD* homology for recombination** Stop *lacI*
181 ***AAGCCTGCAT TGCGGCGCTT CAGTCTCCGC TGCATACTGT CC***TTAATAAA GTGAGTCGAT

241 ATTGTCTTTG TTGACCAGTA ATACCTTATG GAAACGGATA ATTCGCTTAT CCATATCTAC
301 GTCGGCCTTA CCCAGATTCT GCATTTCTAA TCCAGGCTTG ATCTCTTCAC CCTTCAGCAA
361 CGTGCTGGCG ACGGCTGCGA GTGCGTAACC TGCAGAGGCC GGATCGTAAG TAATCCCTTC
421 GGTGATATCA CCACTTTTAA TCAGTGATGC CGCCTGTGAA GGGATCATCA TGCCATAGAC
481 TGCGACTTTA TTTTTCGCCC GTTTCTCTTT CACCGCACGT CCCGCGCCAA TCGGACCGTT
541 TGAACCAAAG GAGACAACCG CTTTCAAGTC AGGATAGGTT TTCATCAGGT CCAGTGTAGT
601 ACGACGTGAG ACATCCACAC TCTCGGCAAC CGGCATGCGG CGGGTAACTT CATGCATATC
661 CGGGTAATGC TCTTTCTGGT ATTTCACCAG CAAGTCAGCC CATAAGTTAT GCTGCGGCAC
721 GGTCAAACTA CCCACGTAAA TCACATAGCC GCCCTTGCCA CCCATGCGTT TCGCCATATG
781 CTCAACATAT TCAGCGGCAA ATTTTTCGTT ATCAATGATT TCGATATCCC AGTTAGCACT
841 TGGCTGACCG GGGGATTCGT TGGTCAGAAC CACAATTCCG GCATCTCGCG CTTTTTTGAA
901 TACCGGTTCC AGCACGTTGG CATCGTTTGG CACGATAGTA ATTGCATTAA CCTTACGGGC
961 GATTAAATCC TCAATAATTT TAACTTGTTG CGGAGCATCA GTACTTGAAG GCCCCACCTG
1021 TGAGGCATTA ACACCAAAGG CTTTACCCGC CTCAACCACA CCTTCGCCCA TGCGATTAAA
1081 CCACGGCATA CCATCGACTT TAGAAATATT CACCACGACT TTTTCCGCTG CCTGGAGCGG
1141 CGCAGAAATT AGCGCAGCGC CTAATAACAG CGAAGACACC ATATTGATAA CAAAACGTTT

Start *lacI* Start *ftsZ*
1201 ATTCAT**CAT** **Ptac sequence (see reference below) A TG**GAACTTAC

12 CAATGACGCG GTGATTAAAG TCATCGGCGT CGGCGGCGGC GGCGGTAATG CTGTTGAACA
72 CATGGTGCGC GAGCGCATTG AAGGTGTTGA ATTCTTCGCG GTAAATACCG ATGCACAAGC
132 GCTGCGTAAA ACAGCGGTTG GACAGACGAT TCAAATCGGT AGCGGTATCA CCAAAGGACT
192 GGGCGCTGGC GCTAATCCAG AAGTTGGCCG CAATGCGGCT GATGAGGATC GCGATGCATT
252 GCGTGCGGCG CTGGAAGGTG CAGACATGGT CTTTATTGCT GCGGGTATGG GTGGTGGTAC
312 CGGTACAGGT GCAGCACCAG TCGTCGCTGA AGTGGCAAAA GATTTGGGTA TCCTGACCGT
372 TGCTGTCGTC ACTAAGCCTT TCAACTTTGA AGGCAAGAAG CGTATGGCAT TCGCGGAGCA
432 GGGGATCACT GAACTGTCCA AGCATGTGGA CTCTCTGATC ACTATCCCGA ACGACAAACT
492 GCTGAAAGTT CTGGGCCGCG GTATCTCCCT GCTGGATGCG TTTGGCGCAG CGAACGATGT
552 ACTGAAAGGC GCTGTGCAAG GTATCGCTGA ACTGATTACT CGTCCGGGTT TGATGAACGT
612 GGACTTTGCA GACGTACGCA CCGTAATGTC TGAGATGGGC TACGCAATGA TGGGTTCTGG
672 CGTGGCGAGC GGTGAAGACC GTGCGGAAGA AGCTGCTGAA ATGGCTATCT CTTCTCCGCT
732 GCTGGAAGAT ATCGACCTGT CTGGCGCGCG CGGCGTGCTG GTTAACATCA CGGCGGGCTT
792 CGACCTGCGT CTGGATGAGT TCGAAACGGT AGGTAACACC ATCCGTGCAT TTGCTTCCGA
852 CAACGCGACT GTGGTTATCG GTACTTCTCT TGACCCGGAT ATGAATGACG AGCTGCGCGT
912 AACCGTTGTT GCGACAGGTA TCGGCATGGA CAAACGTCCT GAAATCACTC TGGTGACCAA
972 TAAGCAGGTT CAGCAGCCAG TGATGGATCG CTACCAGCAG CATGGGATGG CTCCGCTGAC
1032 CCAGGAGCAG AAGCCGGTTG CTAAAGTCGT GAATGACAAT GCGCCGCAAA CTGCGAAAGA
1092 GCCGGATTAT CTGGATATCC CAGCATTCCT GCGTAAGCAA GCTGATTAAT AATCTAGAGG
1152 CGTTACCAAT TATGACAACT TGACGG**GAAG TTCCTATTCT CTAGAAAGTA TAGGAACTTC**
1212 ***CCAAAGCCAG TATCAACTCA GACAAAGGCA AAGCATCTTG***

Bold, italicized represents homology between the PCR product shown below and *intD*.

lacI::Ptac::*ftsZ*::FRT::*kan*::Frt

Following RED recombination into *intD*, the kanamycin cassette was removed with *flp* recombinase resulting in a single FRT scar as depicted above.

Garrido, T., et al. 1993. Transcription of *ftsZ* oscillates during the cell cycle of *Escherichia coli*. EMBO J. 12:3957-3965

SEQ ID NO 6

pMPX-5 expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG
TCAGCGGGTG
121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA
CTGAGAGTGC
181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC
ATCAGGCGCC
241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC
TCTTCGCTAT
301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
ACGCCAGGGT

Stop rhaR
361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GC**TTA**ATTAA
TCTTTCTGCG

421 AATTGAGATG ACGCCACTGG CTGGGCGTCA TCCCGGTTTC CCGGGTAAAC
ACCACCGAAA
481 AATAGTTACT ATCTTCAAAG CCACATTCGG TCGAAATATC ACTGATTAAC
AGGCGGCTAT
541 GCTGGAGAAG ATATTGCGCA TGACACACTC TGACCTGTCG CAGATATTGA
TTGATGGTCA
601 TTCCAGTCTG CTGGCGAAAT TGCTGACGCA AAACGCGCTC ACTGCACGAT
GCCTCATCAC
661 AAAATTTATC CAGCGCAAAG GGACTTTTCA GGCTAGCCGC CAGCCGGGTA
ATCAGCTTAT
721 CCAGCAACGT TTCGCTGGAT GTTGGCGGCA ACGAATCACT GGTGTAACGA
TGGCGATTCA
781 GCAACATCAC CAACTGCCCG AACAGCAACT CAGCCATTTC GTTAGCAAAC
GGCACATGCT
841 GACTACTTTC ATGCTCAAGC TGACCGATAA CCTGCCGCGC CTGCGCCATC
CCCATGCTAC
901 CTAAGCGCCA GTGTGGTTGC CCTGCGCTGG CGTTAAATCC CGGAATCGCC
CCCTGCCAGT
961 CAAGATTCAG CTTCAGACGC TCCGGGCAAT AAATAATATT CTGCAAAACC
AGATCGTTAA
1021 CGGAAGCGTA GGAGTGTTTA TCGTCAGCAT GAATGTAAAA GAGATCGCCA
CGGGTAATGC
1081 GATAAGGGCG ATCGTTGAGT ACATGCAGGC CATTACCGCG CCAGACAATC
ACCAGCTCAC
1141 AAAAATCATG TGTATGTTCA GCAAAGACAT CTTGCGGATA ACGGTCAGCC
ACAGCGACTG
1201 CCTGCTGGTC GCTGGCAAAA AAATCATCTT TGAGAAGTTT TAACTGATGC
GCCACCGTGG
1261 CTACCTCGGC CAGAGAACGA AGTTGATTAT TCGCAATATG GCGTACAAAT
ACGTTGAGAA

Stop rhaS Start rhaR
1321 GATTCGCG**TT A**TTGCAGAAA GC**CAT**CCCGT CCCTGGCGAA TATCACGCGG
TGACCAGTTA
←

1381 AACTCTCGGC GAAAAAGCGT CGAAAAGTGG TTACTGTCGC TGAATCCACA GCGATAGGCG
1441 ATGTCAGTAA CGCTGGCCTC GCTGTGGCGT AGCAGATGTC GGGCTTTCAT CAGTCGCAGG
1501 CGGTTCAGGT ATCGCTGAGG CGTCAGTCCC GTTTGCTGCT TAAGCTGCCG ATGTAGCGTA
1561 CGCAGTGAAA GAGAAAATTG ATCCGCCACG GCATCCCAAT TCACCTCATC GGCAAAATGG
1621 TCCTCCAGCC AGGCCAGAAG CAAGTTGAGA CGTGATGCGC TGTTTTCCAG GTTCTCCTGC
1681 AAACTGCTTT TACGCAGCAA GAGCAGTAAT TGCATAAACA AGATCTCGCG ACTGGCGGTC
1741 GAGGGTAAAT CATTTTCCCC TTCCTGCTGT TCCATCTGTG CAACCAGCTG TCGCACCTGC
1801 TGCAATACGC TGTGGTTAAC GCGCCAGTGA GACGGATACT GCCCATCCAG CTCTTGTGGC
1861 AGCAACTGAT TCAGCCCGGC GAGAAACTGA AATCGATCCG GCGAGCGATA CAGCACATTG
1921 GTCAGACACA GATTATCGGT ATGTTCATAC AGATGCCGAT CATGATCGCG TACGAAACAG
1981 ACCGTGCCAC CGGTGATGGT ATAGGGCTGC CCATTAAACA CATGAATACC CGTGCCATGT
2041 TCGACAATCA CAATTTCATG AAAATCATGA TGATGTTCAG GAAAATCCGC CTGCGGGAGC
2101 CGGGGTTCTA TCGCCACGGA CGCGTTACCA GACGGAAAAA AATCCACACT ATGTAATACG

Start rhaS
2161 GT**CAT**ACTGG *CCTCCTGATG TCGTCAACAC GGCGAAATAG TAATCACGAG GTCAGGTTCT*
←

2221 *TACCTTAAAT TTTCGACGGA AAACCACGTA AAAAACGTCG ATTTTTCAAG ATACAGCGTG*
2281 *AATTTTCAGG AAATGCGGTG AGCATCACAT CACCACAATT CAGCAAATTG TGAACATCAT*
2341 *CACGTTCATC TTTCCCTGGT TGCCAATGGC CCATTTTCCT GTCAGTAACG AGAAGGTCGC*

a. Shine-Delgarno
PstI

2401 *GAATTCAGGC GCTTTTTAGA CTGGTCGTAA TGAAATTC**AG CAGG**ATCACA* TTCTGCAGGT
→

SalI XbaI BamHI KpnI
2461 CGACTCTAGA GGATCCCCGG GTACCGAGCT CGAATTCGTA ATCATGGTCA TAGCTGTTTC
2521 CTGTGTGAAA TTGTTATCCG CTCACAATTC CACACAACAT ACGAGCCGGA AGCATAAAGT
2581 GTAAAGCCTG GGGTGCCTAA TGAGTGAGCT AACTCACATT AATTGCGTTG CGCTCACTGC
2641 CCGCTTTCCA GTCGGGAAAC CTGTCGTGCC AGCTGCATTA ATGAATCGGC CAACGCGCGG
2701 GGAGAGGCGG TTTGCGTATT GGGCGCTCTT CCGCTTCCTC GCTCACTGAC TCGCTGCGCT
2761 CGGTCGTTCG GCTGCGGCGA GCGGTATCAG CTCACTCAAA GGCGGTAATA CGGTTATCCA

2821 CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA
2881 ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC
2941 ACAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG
3001 CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG CTTACCGGAT
3061 ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC TCATAGCTCA CGCTGTAGGT
3121 ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG TGTGCACGAA CCCCCCGTTC
3181 AGCCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG
3241 ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG
3301 GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGG ACAGTATTTG
3361 GTATCTGCGC TCTGCTGAAG CCAGTTACCT TCGGAAAAAG AGTTGGTAGC TCTTGATCCG
3421 GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTTGTTTG CAAGCAGCAG ATTACGCGCA
3481 GAAAAAAAGG ATCTCAAGAA GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA
3541 ACGAAAACTC ACGTTAAGGG ATTTTGGTCA TGAGATTATC AAAAAGGATC TTCACCTAGA
3601 TCCTTTTAAA TTAAAAATGA AGTTTTAAAT CAATCTAAAG TATATATGAG TAAACTTGGT

Stop bla
3661 CTGACAG**TTA** CCAATGCTTA ATCAGTGAGG CACCTATCTC AGCGATCTGT CTATTTCGTT

3721 CATCCATAGT TGCCTGACTC CCCGTCGTGT AGATAACTAC GATACGGGAG GGCTTACCAT
3781 CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG
3841 CAATAAACCA GCCAGCCGGA AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT
3901 CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT
3961 TGCGCAACGT TGTTGCCATT GCTACAGGCA TCGTGGTGTC ACGCTCGTCG TTTGGTATGG
4021 CTTCATTCAG CTCCGGTTCC CAACGATCAA GGCGAGTTAC ATGATCCCCC ATGTTGTGCA
4081 AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT
4141 TATCACTCAT GGTTATGGCA GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT
4201 GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT ATGCGGCGAC
4261 CGAGTTGCTC TTGCCCGGCG TCAATACGGG ATAATACCGC GCCACATAGC AGAACTTTAA
4321 AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT CTCAAGGATC TTACCGCTGT
4381 TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT
4441 TCACCAGCGT TTCTGGGTGA GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA


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                                        Start bla
4501   GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT
TGAAGCATTT
                                           ←

4561   ATCAGGGTTA TTGTCTCATG AGCGGATACA TATTTGAATG TATTTAGAAA
AATAAACAAA
4621   TAGGGGTTCC GCGCACATTT CCCCGAAAAG TGCCACCTGA CGTCTAAGAA
ACCATTATTA
4681   TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC CTTTCGTC
```

The segment *rhaR* through the Prha control region was taken from the *E. coli* MG1655 chromosome using PCR-added *Hin*dIII and *Pst*I restriciton sites. This fragment was cut with *Hin*dIII and *Pst*I and cloned into pUC-18 cut with the same enzymes. Italicized sequence constitutes both *rhaSR* and protein to be expressed promotor region.

SEQ ID NO 7

pMPX-32 (Δ*phoA* cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                                           Shine-Delgarno        PstI
2401   GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
                                                                          M

2461   GCCTGTTCTGGAAAACCGGGCTGCTCAGGGCGATATTACTGCACCCGGCGGTGCTCGCCG
2        P  V  L  E  N  R  A  A  Q  G  D  I  T  A  P  G  G  A  R  R

2521   TTTAACGGGTGATCAGACTGCCGCTCTGCGTGATTCTCTTAGCGATAAACCTGCAAAAAA
22       L  T  G  D  Q  T  A  A  L  R  D  S  L  S  D  K  P  A  K  N

2581   TATTATTTTGCTGATTGGCGATGGGATGGGGGACTCGGAAATTACTGCCGCACGTAATTA
42       I  I  L  L  I  G  D  G  M  G  D  S  E  I  T  A  A  R  N  Y

2641   TGCCGAAGGTGCGGGCGGCTTTTTTAAAGGTATAGATGCCTTACCGCTTACCGGGCAATA
62       A  E  G  A  G  G  F  F  K  G  I  D  A  L  P  L  T  G  Q  Y

2701   CACTCACTATGCGCTGAATAAAAAAACCGGCAAACCGGACTACGTCACCGACTCGGCTGC
82       T  H  Y  A  L  N  K  K  T  G  K  P  D  Y  V  T  D  S  A  A

2761   ATCAGCAACCGCCTGGTCAACCGGTGTCAAAACCTATAACGGCGCGCTGGGCGTCGATAT
102      S  A  T  A  W  S  T  G  V  K  T  Y  N  G  A  L  G  V  D  I

2821   TCACGAAAAAGATCACCCAACGATTCTGGAAATGGCAAAAGCCGCAGGTCTGGCGACCGG
122      H  E  K  D  H  P  T  I  L  E  M  A  K  A  A  G  L  A  T  G

2881   TAACGTTTCTACCGCAGAGTTGCAGGATGCCACGCCCGCTGCGCTGGTGGCACATGTGAC
142      N  V  S  T  A  E  L  Q  D  A  T  P  A  A  L  V  A  H  V  T

2941   CTCGCGCAAATGCTACGGTCCGAGCGCGACCAGTGAAAAATGTCCGGGTAACGCTCTGGA
162      S  R  K  C  Y  G  P  S  A  T  S  E  K  C  P  G  N  A  L  E

3001   AAAAGGCGGAAAAGGATCGATTACCGAACAGCTGCTTAACGCTCGTGCCGACGTTACGCT
182      K  G  G  K  G  S  I  T  E  Q  L  L  N  A  R  A  D  V  T  L

3061   TGGCGGCGGCGCAAAAACCTTTGCTGAAACGGCAACCGCTGGTGAATGGCAGGGAAAAAC
202      G  G  G  A  K  T  F  A  E  T  A  T  A  G  E  W  Q  G  K  T

3121   GCTGCGTGAACAGGCACAGGCGCGTGGTTATCAGTTGGTGAGCGATGCTGCCTCACTGAA
```

```
222        L  R  E  Q  A  Q  A  R  G  Y  Q  L  V  S  D  A  A  S  L  N

3181       TTCGGTGACGGAAGCGAATCAGCAAAAACCCCTGCTTGGCCTGTTTGCTGACGGCAATAT
242         S  V  T  E  A  N  Q  Q  K  P  L  L  G  L  F  A  D  G  N  M

3241       GCCAGTGCGCTGGCTAGGACCGAAAGCAACGTACCATGGCAATATCGATAAGCCCGCAGT
262         P  V  R  W  L  G  P  K  A  T  Y  H  G  N  I  D  K  P  A  V

3301       CACCTGTACGCCAAATCCGCAACGTAATGACAGTGTACCAACCCTGGCGCAGATGACCGA
282         T  C  T  P  N  P  Q  R  N  D  S  V  P  T  L  A  Q  M  T  D

3361       CAAAGCCATTGAATTGTTGAGTAAAAATGAGAAAGGCTTTTTCCTGCAAGTTGAAGGTGC
302         K  A  I  E  L  L  S  K  N  E  K  G  F  F  L  Q  V  E  G  A

3421       GTCAATCGATAAACAGGATCATGCTGCGAATCCTTGTGGGCAAATTGGCGAGACGGTCGA
322         S  I  D  K  Q  D  H  A  A  N  P  C  G  Q  I  G  E  T  V  D

3481       TCTCGATGAAGCCGTACAACGGGCGCTGGAATTCGCTAAAAAGGAGGGTAACACGCTGGT
342         L  D  E  A  V  Q  R  A  L  E  F  A  K  K  E  G  N  T  L  V

3541       CATAGTCACCGCTGATCACGCCCACGCCAGCCAGATTGTTGCGCCGGATACCAAAGCTCC
362         I  V  T  A  D  H  A  H  A  S  Q  I  V  A  P  D  T  K  A  P

3601       GGGCCTCACCCAGGCGCTAAATACCAAAGATGGCGCAGTGATGGTGATGAGTTACGGGAA
382         G  L  T  Q  A  L  N  T  K  D  G  A  V  M  V  M  S  Y  G  N

3661       CTCCGAAGAGGATTCACAAGAACATACCGGCAGTCAGTTGCGTATTGCGGCGTATGGCCC
402         S  E  E  D  S  Q  E  H  T  G  S  Q  L  R  I  A  A  Y  G  P

3721       GCATGCCGCCAATGTTGTTGGACTGACCGACCAGACCGATCTCTTCTACACCATGAAAGC
422         H  A  A  N  V  V  G  L  T  D  Q  T  D  L  F  Y  T  M  K  A

                                          XbaI
3781       CGCTCTGGGGCTGAAATAATAATCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT
442         A  L  G  L  K
```

Δ*phoA* sequence constitutes *phoA* residues 49-453.

SEQ ID NO 8

pMPX-53 (*phoA* cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                                  Shine-Delgarno      PstI
2401       GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
                                                                       M

2461       GTCACGGCCGAGACTTATAGTCGCTTTGTTTTTATTTTTTAATGTATTTGTACATGGAGA
2           S  R  P  R  L  I  V  A  L  F  L  F  F  N  V  F  V  H  G  E

2521       AAATAAAGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGTTACTGTTTACCCCTGT
22          N  K  V  K  Q  S  T  I  A  L  A  L  L  P  L  L  F  T  P  V

2581       GACAAAAGCCCGGACACCAGAAATGCCTGTTCTGGAAAACCGGGCTGCTCAGGGCGATAT
42          T  K  A  R  T  P  E  M  P  V  L  E  N  R  A  A  Q  G  D  I
```



```
2641   TACTGCACCCGGCGGTGCTCGCCGTTTAACGGGTGATCAGACTGCCGCTCTGCGTGATTC
62      T  A  P  G  G  A  R  R  L  T  G  D  Q  T  A  A  L  R  D  S

2701   TCTTAGCGATAAACCTGCAAAAAATATTATTTTGCTGATTGGCGATGGGATGGGGGACTC
82      L  S  D  K  P  A  K  N  I  I  L  L  I  G  D  G  M  G  D  S

2761   GGAAATTACTGCCGCACGTAATTATGCCGAAGGTGCGGGCGGCTTTTTTAAAGGTATAGA
102     E  I  T  A  A  R  N  Y  A  E  G  A  G  G  F  F  K  G  I  D

2821   TGCCTTACCGCTTACCGGGCAATACACTCACTATGCGCTGAATAAAAAAACCGGCAAACC
122     A  L  P  L  T  G  Q  Y  T  H  Y  A  L  N  K  K  T  G  K  P

2881   GGACTACGTCACCGACTCGGCTGCATCAGCAACCGCCTGGTCAACCGGTGTCAAAACCTA
142     D  Y  V  T  D  S  A  A  S  A  T  A  W  S  T  G  V  K  T  Y

2941   TAACGGCGCGCTGGGCGTCGATATTCACGAAAAAGATCACCCAACGATTCTGGAAATGGC
162     N  G  A  L  G  V  D  I  H  E  K  D  H  P  T  I  L  E  M  A

3001   AAAAGCCGCAGGTCTGGCGACCGGTAACGTTTCTACCGCAGAGTTGCAGGATGCCACGCC
182     K  A  A  G  L  A  T  G  N  V  S  T  A  E  L  Q  D  A  T  P

3061   CGCTGCGCTGGTGGCACATGTGACCTCGCGCAAATGCTACGGTCCGAGCGCGACCAGTGA
202     A  A  L  V  A  H  V  T  S  R  K  C  Y  G  P  S  A  T  S  E

3121   AAAATGTCCGGGTAACGCTCTGGAAAAAGGCGGAAAAGGATCGATTACCGAACAGCTGCT
222     K  C  P  G  N  A  L  E  K  G  G  K  G  S  I  T  E  Q  L  L

3181   TAACGCTCGTGCCGACGTTACGCTTGGCGGCGGCGCAAAAACCTTTGCTGAAACGGCAAC
242     N  A  R  A  D  V  T  L  G  G  G  A  K  T  F  A  E  T  A  T

3241   CGCTGGTGAATGGCAGGGAAAAACGCTGCGTGAACAGGCACAGGCGCGTGGTTATCAGTT
262     A  G  E  W  Q  G  K  T  L  R  E  Q  A  Q  A  R  G  Y  Q  L

3301   GGTGAGCGATGCTGCCTCACTGAATTCGGTGACGGAAGCGAATCAGCAAAAACCCCTGCT
282     V  S  D  A  A  S  L  N  S  V  T  E  A  N  Q  Q  K  P  L  L

3361   TGGCCTGTTTGCTGACGGCAATATGCCAGTGCGCTGGCTAGGACCGAAAGCAACGTACCA
302     G  L  F  A  D  G  N  M  P  V  R  W  L  G  P  K  A  T  Y  H

3421   TGGCAATATCGATAAGCCCGCAGTCACCTGTACGCCAAATCCGCAACGTAATGACAGTGT
322     G  N  I  D  K  P  A  V  T  C  T  P  N  P  Q  R  N  D  S  V

3481   ACCAACCCTGGCGCAGATGACCGACAAAGCCATTGAATTGTTGAGTAAAAATGAGAAAGG
342     P  T  L  A  Q  M  T  D  K  A  I  E  L  L  S  K  N  E  K  G

3541   CTTTTTCCTGCAAGTTGAAGGTGCGTCAATCGATAAACAGGATCATGCTGCGAATCCTTG
362     F  F  L  Q  V  E  G  A  S  I  D  K  Q  D  H  A  A  N  P  C

3601   TGGGCAAATTGGCGAGACGGTCGATCTCGATGAAGCCGTACAACGGGCGCTGGAATTCGC
382     G  Q  I  G  E  T  V  D  L  D  E  A  V  Q  R  A  L  E  F  A

3661   TAAAAAGGAGGGTAACACGCTGGTCATAGTCACCGCTGATCACGCCCACGCCAGCCAGAT
402     K  K  E  G  N  T  L  V  I  V  T  A  D  H  A  H  A  S  Q  I

3721   TGTTGCGCCGGATACCAAAGCTCCGGGCCTCACCCAGGCGCTAAATACCAAAGATGGCGC
422     V  A  P  D  T  K  A  P  G  L  T  Q  A  L  N  T  K  D  G  A

3781   AGTGATGGTGATGAGTTACGGGAACTCCGAAGAGGATTCACAAGAACATACCGGCAGTCA
442     V  M  V  M  S  Y  G  N  S  E  E  D  S  Q  E  H  T  G  S  Q
```

```
3841    GTTGCGTATTGCGGCGTATGGCCCGCATGCCGCCAATGTTGTTGGACTGACCGACCAGAC
462      L  R  I  A  A  Y  G  P  H  A  A  N  V  V  G  L  T  D  Q  T

                                                       XbaI
3901    CGATCTCTTCTACACCATGAAAGCCGCTCTGGGGCTGAAATAATCTAGAGGATCCCCGGG
482      D  L  F  Y  T  M  K  A  A  L  G  L  K
```

SEQ ID NO 9

pMPX-33 (*toxR-ΔphoA* cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                            Shine-Delgarno        PstI
2401    GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
                                                                   M

2461    GAACTTGGGGAATCGACTGTTTATTCTGATAGCGGTCTTACTTCCCCTCGCAGTATTACT
2        N  L  G  N  R  L  F  I  L  I  A  V  L  L  P  L  A  V  L  L

2521    GCTCATGCCTGTTCTGGAAAACCGGGCTGCTCAGGGCGATATTACTGCACCCGGCGGTGC
22       L  M  P  V  L  E  N  R  A  A  Q  G  D  I  T  A  P  G  G  A

2581    TCGCCGTTTAACGGGTGATCAGACTGCCGCTCTGCGTGATTCTCTTAGCGATAAACCTGC
42       R  R  L  T  G  D  Q  T  A  A  L  R  D  S  L  S  D  K  P  A

2641    AAAAAATATTATTTTGCTGATTGGCGATGGGATGGGGGACTCGGAAATTACTGCCGCACG
62       K  N  I  I  L  L  I  G  D  G  M  G  D  S  E  I  T  A  A  R

2701    TAATTATGCCGAAGGTGCGGGCGGCTTTTTTAAAGGTATAGATGCCTTACCGCTTACCGG
82       N  Y  A  E  G  A  G  G  F  F  K  G  I  D  A  L  P  L  T  G

2761    GCAATACACTCACTATGCGCTGAATAAAAAAACCGGCAAACCGGACTACGTCACCGACTC
102      Q  Y  T  H  Y  A  L  N  K  K  T  G  K  P  D  Y  V  T  D  S

2821    GGCTGCATCAGCAACCGCCTGGTCAACCGGTGTCAAAACCTATAACGGCGCGCTGGGCGT
122      A  A  S  A  T  A  W  S  T  G  V  K  T  Y  N  G  A  L  G  V

2881    CGATATTCACGAAAAAGATCACCCAACGATTCTGGAAATGGCAAAAGCCGCAGGTCTGGC
142      D  I  H  E  K  D  H  P  T  I  L  E  M  A  K  A  A  G  L  A

2941    GACCGGTAACGTTTCTACCGCAGAGTTGCAGGATGCCACGCCCGCTGCGCTGGTGGCACA
162      T  G  N  V  S  T  A  E  L  Q  D  A  T  P  A  A  L  V  A  H

3001    TGTGACCTCGCGCAAATGCTACGGTCCGAGCGCGACCAGTGAAAAATGTCCGGGTAACGC
182      V  T  S  R  K  C  Y  G  P  S  A  T  S  E  K  C  P  G  N  A

3061    TCTGGAAAAAGGCGGAAAAGGATCGATTACCGAACAGCTGCTTAACGCTCGTGCCGACGT
202      L  E  K  G  G  K  G  S  I  T  E  Q  L  L  N  A  R  A  D  V

3121    TACGCTTGGCGGCGGCGCAAAAACCTTTGCTGAAACGGCAACCGCTGGTGAATGGCAGGG
222      T  L  G  G  G  A  K  T  F  A  E  T  A  T  A  G  E  W  Q  G

3181    AAAAACGCTGCGTGAACAGGCACAGGCGCGTGGTTATCAGTTGGTGAGCGATGCTGCCTC
242      K  T  L  R  E  Q  A  Q  A  R  G  Y  Q  L  V  S  D  A  A  S

3241    ACTGAATTCGGTGACGGAAGCGAATCAGCAAAAACCCCTGCTTGGCCTGTTTGCTGACGG
262      L  N  S  V  T  E  A  N  Q  Q  K  P  L  L  G  L  F  A  D  G
```



```
3301    CAATATGCCAGTGCGCTGGCTAGGACCGAAAGCAACGTACCATGGCAATATCGATAAGCC
282      N  M  P  V  R  W  L  G  P  K  A  T  Y  H  G  N  I  D  K  P

3361    CGCAGTCACCTGTACGCCAAATCCGCAACGTAATGACAGTGTACCAACCCTGGCGCAGAT
302      A  V  T  C  T  P  N  P  Q  R  N  D  S  V  P  T  L  A  Q  M

3421    GACCGACAAAGCCATTGAATTGTTGAGTAAAAATGAGAAAGGCTTTTTCCTGCAAGTTGA
322      T  D  K  A  I  E  L  L  S  K  N  E  K  G  F  F  L  Q  V  E

3481    AGGTGCGTCAATCGATAAACAGGATCATGCTGCGAATCCTTGTGGGCAAATTGGCGAGAC
342      G  A  S  I  D  K  Q  D  H  A  A  N  P  C  G  Q  I  G  E  T

3541    GGTCGATCTCGATGAAGCCGTACAACGGGCGCTGGAATTCGCTAAAAAGGAGGGTAACAC
362      V  D  L  D  E  A  V  Q  R  A  L  E  F  A  K  K  E  G  N  T

3601    GCTGGTCATAGTCACCGCTGATCACGCCCACGCCAGCCAGATTGTTGCGCCGGATACCAA
382      L  V  I  V  T  A  D  H  A  H  A  S  Q  I  V  A  P  D  T  K

3661    AGCTCCGGGCCTCACCCAGGCGCTAAATACCAAAGATGGCGCAGTGATGGTGATGAGTTA
402      A  P  G  L  T  Q  A  L  N  T  K  D  G  A  V  M  V  M  S  Y

3721    CGGGAACTCCGAAGAGGATTCACAAGAACATACCGGCAGTCAGTTGCGTATTGCGGCGTA
422      G  N  S  E  E  D  S  Q  E  H  T  G  S  Q  L  R  I  A  A  Y

3781    TGGCCCGCATGCCGCCAATGTTGTTGGACTGACCGACCAGACCGATCTCTTCTACACCAT
442      G  P  H  A  A  N  V  V  G  L  T  D  Q  T  D  L  F  Y  T  M

                                           XbaI
3841    GAAAGCCGCTCTGGGGCTGAAATAATAATCTAGAGGATCCCCGGGTACCGAGCTCGAATT
462      K  A  A  L  G  L  K
```

Non-bold, underlined sequence is *toxR* transmembrane domain segment that constitutes *toxR* residues 178-198. The remaining sequence is from Δ*phoA* constituting *phoA* residues 49-453.

SEQ ID NO 10

pMPX-7 expression vector

```
1       TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
GAGACGGTCA
61      CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG
TCAGCGGGTG
121     TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA
CTGAGAGTGC
181     ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC
ATCAGGCGCC
241     ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC
TCTTCGCTAT
301     TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
ACGCCAGGGT

                                                 HindIII
361     TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTCGCAGC
GCTGTTCCTT
```

421 TGCTCGCCTG CTGCGAGCTG GGTAAGCGGA CAAATTCTCA CCGTCTCCGG TGGTGGGGTA
481 CAGGAGCTCA ATTAATACAC TAACGGACCG GTAAACAACC GTGCGTGTTG TTTACCGGGA
541 TAAACTCATC AACGTCTCTG CTAAATAACT GGCAGCCAAA TCACGGCTAT TGGTTAACCA
601 ATTTCAGAGT GAAAAGTATA CGAATAGAGT GTGCCTTCGC ACTATTCAAC AGCAATGATA

Start uidR
661 GGCGCTCACC TGACAACGCG GTAAACTAGT TATTCACGCT AACTATAATG GTTTA**ATG**AT →

721 GGATAACATG CAGACTGAAG CACAACCGAC ACGGACCCGG ATCCTCAATG CTGCCAGAGA
781 GATTTTTTCA GAAAATGGAT TTCACAGTGC CTCGATGAAA GCCATCTGTA AATCTTGCGC
841 CATTAGTCCC GGGACGCTCT ATCACCATTT CATCTCCAAA GAAGCCTTGA TTCAGGCGAT
901 TATCTTACAG GACCAGGAGA GGGCGCTGGC CCGTTTCCGG GAACCGATTG AAGGGATTCA
961 TTTCGTTGAC TATATGGTCG AGTCCATTGT CTCTCTCACC CATGAAGCCT TTGGACAACG
1021 GGCGCTGGTG GTTGAAATTA TGGCGGAAGG GATGCGTAAC CCACAGGTCG CCGCCATGCT
1081 TAAAAATAAG CATATGACGA TCACGGAATT TGTTGCCCAG CGGATGCGTG ATGCCCAGCA
1141 AAAAGGCGAG ATAAGCCCAG ACATCAACAC GGCAATGACT TCACGTTTAC TGCTGGATCT
1201 GACCTACGGT GTACTGGCCG ATATCGAAGC GGAAGACCTG GCGCGTGAAG CGTCGTTTGC

Stop uidR
1261 TCAGGGATTA CGCGCGATGA TTGGCGGTAT CTTAACCGCA TCC**TGA**TTCT *CTCTCTTTTT*

1321 *CGGCGGGCTG GTGATAACTG TGCCCGCGTT TCATATCGTA ATTTCTCTGT GCAAAAATTA*
1381 TCCTTCCCGG CTTCGGAGAA TTCCCCCCAA AATATTCACT GTAGCCATAT GTCATGAGAG
1441 *TTTATCGTTC CCAATACGCT CGAACGAACG TTCGGTTGCT TATTTTATGG CTTCTGTCAA*
1501 *CGCTGTTTTA AAGATTAATG CGATCTATAT CACGCTGTGG GTATTGCAGT TTTTGGTTTT*
1561 *TTGATCGCGG TGTCAGTTCT TTTTATTTCC ATTTCTCTTC CATGGGTTTC TCACAGATAA*
1621 *CTGTGTGCAA CACAGAATTG GTTAACTAAT CAGATTAAAG GTTGACCAGT ATTATTATCT*

Shine-Delgarno PstI SalI XbaI KpnI
1681 *TA**ATGAGG**AG* TCCTGCAGGT CGACTCTAGA GGATCCCCGG GTACCGAGCT CGAATTCGTA →

1741 ATCATGGTCA TAGCTGTTTC CTGTGTGAAA TTGTTATCCG CTCACAATTC CACACAACAT
1801 ACGAGCCGGA AGCATAAAGT GTAAAGCCTG GGGTGCCTAA TGAGTGAGCT AACTCACATT

1861 AATTGCGTTG CGCTCACTGC CCGCTTTCCA GTCGGGAAAC CTGTCGTGCC AGCTGCATTA
1921 ATGAATCGGC CAACGCGCGG GGAGAGGCGG TTTGCGTATT GGGCGCTCTT CCGCTTCCTC
1981 GCTCACTGAC TCGCTGCGCT CGGTCGTTCG GCTGCGGCGA GCGGTATCAG CTCACTCAAA
2041 GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA TGTGAGCAAA
2101 AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG CTGGCGTTTT TCCATAGGCT
2161 CCGCCCCCCT GACGAGCATC ACAAAAATCG ACGCTCAAGT CAGAGGTGGC GAAACCCGAC
2221 AGGACTATAA AGATACCAGG CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC
2281 GACCCTGCCG CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC
2341 TCATAGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA AGCTGGGCTG
2401 TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA TCCGGTAACT ATCGTCTTGA
2461 GTCCAACCCG GTAAGACACG ACTTATCGCC ACTGGCAGCA GCCACTGGTA ACAGGATTAG
2521 CAGAGCGAGG TATGTAGGCG GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA
2581 CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT TCGGAAAAAG
2641 AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT TTTTTGTTTG
2701 CAAGCAGCAG ATTACGCGCA GAAAAAAAGG ATCTCAAGAA GATCCTTTGA TCTTTTCTAC
2761 GGGGTCTGAC GCTCAGTGGA ACGAAAACTC ACGTTAAGGG ATTTTGGTCA TGAGATTATC
2821 AAAAAGGATC TTCACCTAGA TCCTTTTAAA TTAAAAATGA AGTTTTAAAT CAATCTAAAG

Stop bla
2881 TATATATGAG TAAACTTGGT CTGACAG**TTA** CCAATGCTTA ATCAGTGAGG CACCTATCTC

2941 AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC CCCGTCGTGT AGATAACTAC
3001 GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG ATACCGCGAG ACCCACGCTC
3061 ACCGGCTCCA GATTTATCAG CAATAAACCA GCCAGCCGGA AGGGCCGAGC GCAGAAGTGG
3121 TCCTGCAACT TTATCCGCCT CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG
3181 TAGTTCGCCA GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTACAGGCA TCGTGGTGTC
3241 ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC CAACGATCAA GGCGAGTTAC
3301 ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC GGTCCTCCGA TCGTTGTCAG
3361 AAGTAAGTTG GCCGCAGTGT TATCACTCAT GGTTATGGCA GCACTGCATA ATTCTCTTAC
3421 TGTCATGCCA TCCGTAAGAT GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG
3481 AGAATAGTGT ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAATACGGG ATAATACCGC

3541 GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG GGCGAAAACT
3601 CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA CCCACTCGTG CACCCAACTG
3661 ATCTTCAGCA TCTTTTACTT TCACCAGCGT TTCTGGGTGA GCAAAAACAG GAAGGCAAAA

Start bla
3721 TGCCGCAAAA AAGGGAATAA GGGCGACACG GAAATGTTGA ATACT**CAT**AC TCTTCCTTTT
←

3781 TCAATATTAT TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA TATTTGAATG
3841 TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG TGCCACCTGA
3901 CGTCTAAGAA ACCATTATTA TCATGACATT AACCTATAAA AATAGGCGTA TCACGAGGCC
3961 CTTTCGTC

The segment *uidR control region* through the Puid promotor region was taken from the *E. coli* MG1655 chromosome using PCR-added *Hin*dIII and *Pst*I restriciton sites. This fragment was cut with *Hin*dIII and *Pst*I and cloned into pUC-18 cut with the same enzymes. Underlined sequence constitutes the *uidR* regulatory region while the italicized sequence constitutes the protein to be expressed promotor region under the control of *uidR*.

SEQ ID NO 11

pMPX-8 expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC
241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT
301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT

Stop melR
361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTT**TTA**GCC GGGAAACGTC

421 TGGCGGCGCT GTTGGCTAAG TTTGCGGTAT TGTTGCGGCG ACATGCCGAC ATATTTGCCG
481 AACGTGCTGT AAAAACGACT ACTTGAACGA AAGCCTGCCG TCAGGGCAAT ATCGAGAATA
541 CTTTTATCGG TATCGCTCAG TAACGCGCGA ACGTGGTTGA TGCGCATCGC GGTAATGTAC
601 TGTTTCATCG TCAATTGCAT GACCCGCTGG AATATCCCCA TTGCATAGTT GGCGTTAAGT

661 TTGACGTGCT CAGCCACATC GTTGATGGTC AGCGCCTGAT CATAGTTTTC GGCAATAAAG
721 CCCAGCATCT GGCTAACATA AAATTGCGCA TGGCGCGAGA CGCTGTTTTT GTGTGTGCGC
781 GAGGTTTTAT TGACCAGAAT CGGTTCCCAG CCAGAGAGGC TAAATCGCTT GAGCATCAGG
841 CCAATTTCAT CAATGGCGAG CTGGCGAATT TGCTCGTTCG GACTGTTTAA TTCCTGCTGC
901 CAGCGGCGCA CTTCAAACGG GCTAAGTTGC TGTGTGGCCA GTGATTTGAT CACCATGCCG
961 TGAGTGACGT GGTTAATCAG GTCTTTATCC AGCGGCCAGG AGAGAAACAG ATGCATCGGC
1021 AGATTAAAAA TCGCCATGCT CTGACAGGTT CCGGTATCTG TTAGTTGGTG CGGTGTACAG
1081 GCCCAGAACA GCGTGATATG ACCCTGATTG ATATTCACTT TTTCATTGTT GATCAGGTAT
1141 TCCACATCGC CATCGAAAGG CACATTCACT TCGACCTGAC CATGCCAGTG GCTGGTGGGC
1201 ATGATATGCG GTGCGCGAAA CTCAATCTCC ATCCGCTGGT ATTCCGAATA CAGCGACAGC

Start melR

1261 GGGCTGCGGG TCTGTTTTTC GTCGCTGCTG CACATAAACG TATCTGTATT <u>**CAT**</u>GGATGGC

←

1321 *TCTCTTTCCT GGAATATCAG AATTATGGCA GGAGTGAGGG AGGATGACTG CGAGTGGGAG*
1381 *CACGGTTTTC ACCCTCTTCC CAGAGGGGCG AGGGGACTCT CCGAGTATCA TGAGGCCGAA*
1441 AACTCTGCTT TTCAGGTAAT TTATTCCCAT AAACTCAGAT TTACTGCTGC TTCACGCAGG

Shine-Delgarno PstI

1501 *ATCTGAGTTT ATGGGAATGC TCAACCTGGA AGC<u>**CGGAGG**</u>T* TTTCTGCAGA TTCGCCTGCC

SalI XbaI

BamHI
1561 ATGATGAAGT TATTCAAGCA AGCCAGGAGA TCTGGTACCC GGGTCGACTC TAGAGGATCC

KpnI
1621 CCGGGTACCG AGCTCGAATT CGTAATCATG GTCATAGCTG TTTCCTGTGT GAAATTGTTA
1681 TCCGCTCACA ATTCCACACA ACATACGAGC CGGAAGCATA AAGTGTAAAG CCTGGGGTGC
1741 CTAATGAGTG AGCTAACTCA CATTAATTGC GTTGCGCTCA CTGCCCGCTT TCCAGTCGGG
1801 AAACCTGTCG TGCCAGCTGC ATTAATGAAT CGGCCAACGC GCGGGGAGAG GCGGTTTGCG
1861 TATTGGGCGC TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTCG TTCGGCTGCG
1921 GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA TCCACAGAAT CAGGGGATAA
1981 CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC AGGAACCGTA AAAAGGCCGC
2041 GTTGCTGGCG TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC

2101 AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG
2161 CTCCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT
2221 CCCTTCGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA GTTCGGTGTA
2281 GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC
2341 CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC
2401 AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT
2461 GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA TTTGGTATCT GCGCTCTGCT
2521 GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC AAACCACCGC
2581 TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG CGCAGAAAAA AAGGATCTCA
2641 AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG TGGAACGAAA ACTCACGTTA
2701 AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA

Stop bla
2761 ATGAAGTTTT AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA G**TTA**CCAATG

2821 CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG
2881 ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
2941 AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA TCAGCAATAA ACCAGCCAGC
3001 CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA
3061 TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC
3121 CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG
3181 TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
3241 CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA GTGTTATCAC TCATGGTTAT
3301 GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG
3361 TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC
3421 GGCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG
3481 AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
3541 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA GCGTTTCTGG
3601 GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG

Start bla
3661 TTGAATACT**C AT**ACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT

←

3721 CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG
TTCCGCGCAC
3781 ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA
CATTAACCTA
3841 TAAAAATAGG CGTATCACGA GGCCCTTTCG TC

The segment *melR* through the Pmel control region was taken from the *E. coli* MG1655 chromosome using PCR-added *Hin*dIII and *Pst*I restriciton sites. This fragment was cut with *Hin*dIII and *Pst*I and cloned into pUC-18 cut with the same enzymes. Italicized sequence constitutes both *melR* and protein to be expressed promotor region.

SEQ ID NO 12

pMPX-18 expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG
GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG
TCAGCGGGTG
121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA
CTGAGAGTGC
181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC
ATCAGGCGCC
241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC
TCTTCGCTAT
301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA
ACGCCAGGGT

HindIII
361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTCAAGCC
GTCAATTGTC

Stop araC
421 TGATTCGTTA CCAATTATGA CAACTTGACG GCTACATCAT TCACTTTTTC
TTCACAACCG

481 GCACGGAACT CGCTCGGGCT GGCCCCGGTG CATTTTTTAA ATACCCGCGA
GAAATAGAGT
541 TGATCGTCAA AACCAACATT GCGACCGACG GTGGCGATAG GCATCCGGGT
GGTGCTCAAA
601 AGCAGCTTCG CCTGGCTGAT ACGTTGGTCC TCGCGCCAGC TTAAGACGCT
AATCCCTAAC
661 TGCTGGCGGA AAAGATGTGA CAGACGCGAC GGCGACAAGC AAACATGCTG
TGCGACGCTG
721 GCGATATCAA AATTGCTGTC TGCCAGGTGA TCGCTGATGT ACTGACAAGC
CTCGCGTACC
781 CGATTATCCA TCGGTGGATG GAGCGACTCG TTAATCGCTT CCATGCGCCG
CAGTAACAAT
841 TGCTCAAGCA GATTTATCGC CAGCAGCTCC GAATAGCGCC CTTCCCCTTG
CCCGGCGTTA
901 ATGATTTGCC CAAACAGGTC GCTGAAATGC GGCTGGTGCG CTTCATCCGG
GCGAAAGAAC
961 CCCGTATTGG CAAATATTGA CGGCCAGTTA AGCCATTCAT GCCAGTAGGC
GCGCGGACGA

1021 AAGTAAACCC ACTGGTGATA CCATTCGCGA GCCTCCGGAT GACGACCGTA GTGATGAATC
1081 TCTCCTGGCG GGAACAGCAA AATATCACCC GGTCGGCAAA CAAATTCTCG TCCCTGATTT
1141 TTCACCACCC CCTGACCGCG AATGGTGAGA TTGAGAATAT AACCTTTCAT TCCCAGCGGT
1201 CGGTCGATAA AAAAATCGAG ATAACCGTTG GCCTCAATCG GCGTTAAACC CGCCACCAGA
1261 TGGGCATTAA ACGAGTATCC CGGCAGCAGG GGATCATTTT GCGCTTCAGC CATACTTTTC

Start araC
1321 ATACTCCCGC CATTCAGAGA AGAAACCAAT TGTCCATATT G**<u>CAT</u>**CAGACA *TTGCCGTCAC*

←

1381 TGCGTCTTTT ACTGGCTCTT CTCGCTAACC AAACCGGTAA CCCCGCTTAT TAAAAGCATT
1441 CTGTAACAAA GCGGGACCAA AGCCATGACA AAAACGCGTA ACAAAAGTGT CTATAATCAC
1501 GGCAGAAAAG TCCACATTGA TTATTTGCAC GGCGTCACAC TTTGCTATGC CATAGCATTT
1561 TTATCCATAA GATTAGCGGA TCCTACCTGA CGCTTTTTAT CGCAACTCTC TACTGTTTCT

Shine-Delgarno **<u>PstI</u>** SalI XbaI
1621 *CCATACCCGT TTTTTTGGGC TAGC**<u>AGGAGG</u>*** AATTCACC**<u>CT GCAG</u>**GTCGAC TCTAGAGGAT

→

XmaI KpnI
1681 CCCCGGGTAC CGAGCTCGAA TTCGTAATCA TGGTCATAGC TGTTTCCTGT GTGAAATTGT
1741 TATCCGCTCA CAATTCCACA CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT
1801 GCCTAATGAG TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG
1861 GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG
1921 CGTATTGGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG
1981 CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT
2041 AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC
2101 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC
2161 TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA
2221 AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT
2281 CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG
2341 TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC
2401 GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG
2461 GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC
2521 TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG

2581 CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC
2641 GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT
2701 CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT
2761 TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA

Start bla
2821 AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA

2881 TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC
2941 TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT
3001 GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA
3061 GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT
3121 AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT
3181 GCCATTGCTA CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC
3241 GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC
3301 TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT
3361 ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT
3421 GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC
3481 CCGGCGTCAA TACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT
3541 GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG
3601 ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT
3661 GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA

Start bla
3721 TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT
←

3781 CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC
3841 ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC
3901 TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTC

The segment *araC* through the Para control region was taken from pBAD24 using PCR-added *Hin*dIII and *Pst*I restriciton sites. This fragment was cut with *Hin*dIII and *Pst*I and cloned into pUC-18 cut with the same enzymes. Italicized sequence constitutes both *araC* and protein to be expressed promotor region.

SEQ ID NO 13

pMPX-6 expression vector

```
1       TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
TGGAGTTCCG
61      CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC
CCCGCCCATT
121     GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC
ATTGACGTCA
181     ATGGGTGGAG TATTTACGGT AAACTGCCCA CTTGGCAGTA CATCAAGTGT
ATCATATGCC
241     AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT
ATGCCCAGTA
301     CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
TCGCTATTAC
361     CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG
ACTCACGGGG
421     ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC
AAAATCAACG
481     GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG
GTAGGCGTGT
541     ACGGTGGGAG GTCTATATAA GCAGAGCTGG TTTAGTGAAC CGTCAGATCC
GCTAGCGCTA

                 Start GFP
601     CCGGTCGCCA CCATGGTGAG CAAGGGCGAG GAGCTGTTCA CCGGGGTGGT
GCCCATCCTG
                   →

661     GTCGAGCTGG ACGGCGACGT AAACGGCCAC AAGTTCAGCG TGTCCGGCGA
GGGCGAGGGC
721     GATGCCACCT ACGGCAAGCT GACCCTGAAG TTCATCTGCA CCACCGGCAA
GCTGCCCGTG
781     CCCTGGCCCA CCCTCGTGAC CACCCTGACC TACGGCGTGC AGTGCTTCAG
CCGCTACCCC
841     GACCACATGA AGCAGCACGA CTTCTTCAAG TCCGCCATGC CCGAAGGCTA
CGTCCAGGAG
901     CGCACCATCT TCTTCAAGGA CGACGGCAAC TACAAGACCC GCGCCGAGGT
GAAGTTCGAG
961     GGCGACACCC TGGTGAACCG CATCGAGCTG AAGGGCATCG ACTTCAAGGA
GGACGGCAAC
1021    ATCCTGGGGC ACAAGCTGGA GTACAACTAC AACAGCCACA ACGTCTATAT
CATGGCCGAC
1081    AAGCAGAAGA ACGGCATCAA GGTGAACTTC AAGATCCGCC ACAACATCGA
GGACGGCAGC
1141    GTGCAGCTCG CCGACCACTA CCAGCAGAAC ACCCCCATCG GCGACGGCCC
CGTGCTGCTG
1201    CCCGACAACC ACTACCTGAG CACCCAGTCC GCCCTGAGCA AAGACCCCAA
CGAGAAGCGC
1261    GATCACATGG TCCTGCTGGA GTTCGTGACC GCCGCCGGGA TCACTCTCGG
CATGGACGAG

                                       XhoI  Stop GFP
1321    CTGTACAAGT CCGGACTCAG ATCTCGAGCT TAATAACAAG CCGTCAATTG
TCTGATTCGT
```


Stop araC
1381 TACCAA**TTA**T GACAACTTGA CGGCTACATC ATTCACTTTT TCTTCACAAC CGGCACGGAA

1441 CTCGCTCGGG CTGGCCCCGG TGCATTTTTT AAATACCCGC GAGAAATAGA GTTGATCGTC
1501 AAAACCAACA TTGCGACCGA CGGTGGCGAT AGGCATCCGG GTGGTGCTCA AAAGCAGCTT
1561 CGCCTGGCTG ATACGTTGGT CCTCGCGCCA GCTTAAGACG CTAATCCCTA ACTGCTGGCG
1621 GAAAAGATGT GACAGACGCG ACGGCGACAA GCAAACATGC TGTGCGACGC TGGCGATATC
1681 AAAATTGCTG TCTGCCAGGT GATCGCTGAT GTACTGACAA GCCTCGCGTA CCCGATTATC
1741 CATCGGTGGA TGGAGCGACT CGTTAATCGC TTCCATGCGC CGCAGTAACA ATTGCTCAAG
1801 CAGATTTATC GCCAGCAGCT CCGAATAGCG CCCTTCCCCT TGCCCGGCGT TAATGATTTG
1861 CCCAAACAGG TCGCTGAAAT GCGGCTGGTG CGCTTCATCC GGGCGAAAGA ACCCCGTATT
1921 GGCAAATATT GACGGCCAGT TAAGCCATTC ATGCCAGTAG GCGCGCGGAC GAAAGTAAAC
1981 CCACTGGTGA TACCATTCGC GAGCCTCCGG ATGACGACCG TAGTGATGAA TCTCTCCTGG
2041 CGGGAACAGC AAAATATCAC CCGGTCGGCA AACAAATTCT CGTCCCTGAT TTTTCACCAC
2101 CCCCTGACCG CGAATGGTGA GATTGAGAAT ATAACCTTTC ATTCCCAGCG GTCGGTCGAT
2161 AAAAAAATCG AGATAACCGT TGGCCTCAAT CGGCGTTAAA CCCGCCACCA GATGGGCATT
2221 AAACGAGTAT CCCGGCAGCA GGGGATCATT TTGCGCTTCA GCCATACTTT TCATACTCCC

Start araC
2281 GCCATTCAGA GAAGAAACCA ATTGTCCATA TTG**CAT**CAGA *CATTGCCGTC ACTGCGTCTT*

←
2341 *TTACTGGCTC TTCTCGCTAA CCAAACCGGT AACCCCGCTT ATTAAAAGCA TTCTGTAACA*
2401 *AAGCGGGACC AAAGCCATGA CAAAAACGCG TAACAAAAGT GTCTATAATC ACGGCAGAAA*
2461 *AGTCCACATT GATTATTTGC ACGGCGTCAC ACTTTGCTAT GCCATAGCAT TTTTATCCAT*
2521 *AAGATTAGCG GATCCTACCT GACGCTTTTT ATCGCAACTC TCTACTGTTT CTCCATACCC*

→ EcoRI KpnI SalI
2581 *GTTTTTTTGG GCTAGC**AGGA GG**AATTCACC* ATGGTACCCG GGGATCCTCT AGAGTCGACC
Shine-Delgarno

PstI HindIII **SstII**
2641 TGCAGGCATG CAAGCTTGGC **CCGCGG**GCCC GGGATCCACC GGATCTAGAT AACTGATCAT

2701 AATCAGCCAT ACCACATTTG TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC
2761 CCTGAACCTG AAACATAAAA TGAATGCAAT TGTTGTTGTT AACTTGTTTA TTGCAGCTTA

2821 TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTTCACA AATAAAGCAT TTTTTTCACT

2881 GCATTCTAGT TGTGGTTTGT CCAAACTCAT CAATGTATCT TAACGCGTAA ATTGTAAGCG

2941 TTAATATTTT GTTAAAATTC GCGTTAAATT TTTGTTAAAT CAGCTCATTT TTTAACCAAT

3001 AGGCCGAAAT CGGCAAAATC CCTTATAAAT CAAAAGAATA GACCGAGATA GGGTTGAGTG

3061 TTGTTCCAGT TTGGAACAAG AGTCCACTAT TAAAGAACGT GGACTCCAAC GTCAAAGGGC

3121 GAAAAACCGT CTATCAGGGC GATGGCCCAC TACGTGAACC ATCACCCTAA TCAAGTTTTT

3181 TGGGGTCGAG GTGCCGTAAA GCACTAAATC GGAACCCTAA AGGGAGCCCC CGATTTAGAG

3241 CTTGACGGGG AAAGCCGGCG AACGTGGCGA GAAAGGAAGG GAAGAAAGCG AAAGGAGCGG

3301 GCGCTAGGGC GCTGGCAAGT GTAGCGGTCA CGCTGCGCGT AACCACCACA CCCGCCGCGC

3361 TTAATGCGCC GCTACAGGGC GCGTCAGGTG GCACTTTTCG GGGAAATGTG CGCGGAACCC

3421 CTATTTGTTT ATTTTTCTAA ATACATTCAA ATATGTATCC GCTCATGAGA CAATAACCCT

3481 GATAAATGCT TCAATAATAT TGAAAAAGGA AGAGTCCTGA GGCGGAAAGA ACCAGCTGTG

3541 GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA

3601 AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG

3661 CAGAAGTATG CAAAGCATGC ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC

3721 GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT

3781 TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG

3841 AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAGATCGA TCAAGAGACA GGATGAGGAT

Start Kan

3901 CGTTTCGC**<u>AT G</u>**ATTGAACAA GATGGATTGC ACGCAGGTTC TCCGGCCGCT TGGGTGGAGA

→

3961 GGCTATTCGG CTATGACTGG GCACAACAGA CAATCGGCTG CTCTGATGCC GCCGTGTTCC

4021 GGCTGTCAGC GCAGGGGCGC CCGGTTCTTT TTGTCAAGAC CGACCTGTCC GGTGCCCTGA

4081 ATGAACTGCA AGACGAGGCA GCGCGGCTAT CGTGGCTGGC CACGACGGGC GTTCCTTGCG

4141 CAGCTGTGCT CGACGTTGTC ACTGAAGCGG GAAGGGACTG GCTGCTATTG GGCGAAGTGC

4201 CGGGGCAGGA TCTCCTGTCA TCTCACCTTG CTCCTGCCGA GAAAGTATCC ATCATGGCTG

4261 ATGCAATGCG GCGGCTGCAT ACGCTTGATC CGGCTACCTG CCCATTCGAC CACCAAGCGA

4321 AACATCGCAT CGAGCGAGCA CGTACTCGGA TGGAAGCCGG TCTTGTCGAT CAGGATGATC

4381 TGGACGAAGA GCATCAGGGG CTCGCGCCAG CCGAACTGTT CGCCAGGCTC AAGGCGAGCA

4441 TGCCCGACGG CGAGGATCTC GTCGTGACCC ATGGCGATGC CTGCTTGCCG AATATCATGG


```
4501    TGGAAAATGG CCGCTTTTCT GGATTCATCG ACTGTGGCCG GCTGGGTGTG
GCGGACCGCT
4561    ATCAGGACAT AGCGTTGGCT ACCCGTGATA TTGCTGAAGA GCTTGGCGGC
GAATGGGCTG
4621    ACCGCTTCCT CGTGCTTTAC GGTATCGCCG CTCCCGATTC GCAGCGCATC
GCCTTCTATC

                               Stop Kan
4681    GCCTTCTTGA CGAGTTCTTC TGAGCGGGAC TCTGGGGTTC GAAATGACCG
ACCAAGCGAC

4741    GCCCAACCTG CCATCACGAG ATTTCGATTC CACCGCCGCC TTCTATGAAA
GGTTGGGCTT
4801    CGGAATCGTT TTCCGGGACG CCGGCTGGAT GATCCTCCAG CGCGGGGATC
TCATGCTGGA
4861    GTTCTTCGCC CACCCTAGGG GGAGGCTAAC TGAAACACGG AAGGAGACAA
TACCGGAAGG
4921    AACCCGCGCT ATGACGGCAA TAAAAAGACA GAATAAAACG CACGGTGTTG
GGTCGTTTGT
4981    TCATAAACGC GGGGTTCGGT CCCAGGGCTG GCACTCTGTC GATACCCCAC
CGAGACCCCA
5041    TTGGGGCCAA TACGCCCGCG TTTCTTCCTT TTCCCCACCC CACCCCCCAA
GTTCGGGTGA
5101    AGGCCCAGGG CTCGCAGCCA ACGTCGGGGC GGCAGGCCCT GCCATAGCCT
CAGGTTACTC
5161    ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT
AGGTGAAGAT
5221    CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG TTTTCGTTCC
ACTGAGCGTC
5281    AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC
GCGTAATCTG
5341    CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTTGCCGG
ATCAAGAGCT
5401    ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA
ATACTGTCCT
5461    TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC
CTACATACCT
5521    CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT
GTCTTACCGG
5581    GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA
CGGGGGGTTC
5641    GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC
TACAGCGTGA
5701    GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC
CGGTAAGCGG
5761    CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT
GGTATCTTTA
5821    TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTTGTGAT
GCTCGTCAGG
5881    GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC
TGGCCTTTTG
5941    CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG
ATAACCGTAT
6001    TACCGCCATG CAT
```

The segment *araC* through *Sst*II following the Para control region was taken from pBAD24 using a PCR-added *Xho*I restriciton site. This fragment was cut with *Xho*I and *SstI*I and cloned into pEGFP-C1 (Clontech) cut with the same enzymes. Italicized and underlined

sequence constitutes the CMV promotor region while the italicized alone region constitutes both the *araC* and protein to be expressed promotor region.

SEQ ID NO 14

pMPX-56 (rat Edg3 cloned into pMPX-5 using PCR-introduced SalI and KpnI)

Shine-Delgarno
2401 GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTC**AGCAGG**ATCACATTCTGCAG**GT**

SalI
2461 **CGAC**ATGGCAACCACGCACGCGCAGGGCCACCCGCCAGTCTTGGGGAATGATACTCTCCG
1 M A T T H A Q G H P P V L G N D T L R

2521 GGAACATTATGATTACGTGGGGAAGCTGGCAGGCAGGCTGCGGGATCCCCCTGAGGGTAG
20 E H Y D Y V G K L A G R L R D P P E G S

2581 CACCCTCATCACCACCATCCTCTTCTTGGTCACCTGTAGCTTCATCGTCTTGGAGAACCT
40 T L I T T I L F L V T C S F I V L E N L

2641 GATGGTTTTGATTGCCATCTGGAAAAACAATAAATTTCATAACCGCATGTACTTTTTCAT
60 M V L I A I W K N N K F H N R M Y F F I

2701 CGGCAACTTGGCTCTCTGCGACCTGCTGGCCGGCATAGCCTACAAGGTCAACATTCTGAT
80 G N L A L C D L L A G I A Y K V N I L M

2761 GTCCGGTAGGAAGACGTTCAGCCTGTCTCCAACAGTGTGGTTCCTCAGGGAGGGCAGTAT
100 S G R K T F S L S P T V W F L R E G S M

2821 GTTCGTAGCCCTGGGCGCATCCACATGCAGCTTATTGGCCATTGCCATTGAGCGGCACCT
120 F V A L G A S T C S L L A I A I E R H L

2881 GACCATGATCAAGATGAGGCCGTACGACGCCAACAAGAAGCACCGCGTGTTCCTTCTGAT
140 T M I K M R P Y D A N K K H R V F L L I

2941 TGGGATGTGCTGGCTAATTGCCTTCTCGCTGGGTGCCCTGCCCATCCTGGGCTGGAACTG
160 G M C W L I A F S L G A L P I L G W N C

3001 CCTGGAAAACTTTCCCGACTGCTCTACCATCTTGCCCCTCTACTCCAAGAAATACATTGC
180 L E N F P D C S T I L P L Y S K K Y I A

3061 CTTTCTCATCAGCATCTTCATAGCCATTCTGGTGACCATCGTCATCTTGTACGCGCGCAT
200 F L I S I F I A I L V T I V I L Y A R I

3121 CTACTTCCTGGTCAAGTCCAGCAGCCGCAGGGTGGCCAACCACAACTCCGAGAGATCCAT
220 Y F L V K S S S R R V A N H N S E R S M

3181 GGCCCTTCTGCGGACCGTAGTGATCGTGGTGAGCGTGTTCATCGCCTGTTGGTCCCCCCT
240 A L L R T V V I V V S V F I A C W S P L

3241 TTTCATCCTCTTCCTCATCGATGTGGCCTGCAGGGCGAAGGAGTGCTCCATCCTCTTCAA
260 F I L F L I D V A C R A K E C S I L F K

```
3301   GAGTCAGTGGTTCATCATGCTGGCTGTCCTCAACTCGGCCATGAACCCTGTCATCTACAC
280     S  Q  W  F  I  M  L  A  V  L  N  S  A  M  N  P  V  I  Y  T

3361   GCTGGCCAGCAAAGAGATGCGGCGTGCTTTCTTCCGGTTGGTGTGCGGCTGTCTGGTCAA
300     L  A  S  K  E  M  R  R  A  F  F  R  L  V  C  G  C  L  V  K

3421   GGGCAAGGGGACCCAGGCCTCCCCGATGCAGCCTGCTCTTGACCCGAGCAGAAGTAAATC
320     G  K  G  T  Q  A  S  P  M  Q  P  A  L  D  P  S  R  S  K  S

3481   AAGCTCCAGTAACAACAGCAGCAGCCACTCTCCAAAGGTCAAGGAAGACCTGCCCCATGT
340     S  S  S  N  N  S  S  S  H  S  P  K  V  K  E  D  L  P  H  V

3541   GGCTACCTCTTCCTGCGTTACTGACAAAACGAGGTCGCTTCAGAATGGGGTCCTCTGCAA
360     A  T  S  S  C  V  T  D  K  T  R  S  L  Q  N  G  V  L  C  K

3601   GAAGGGCAATTCTGCAGATATCCAGCACAGTGGCGGCCGCTCGAGTCTAGAGGGCCCGCG
380     K  G  N  S  A  D  I  Q  H  S  G  G  R  S  S  L  E  G  P  R

3661   GTTCGAAGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGCGTACCGGTCA
400     F  E  G  K  P  I  P  N  P  L  L  G  L  D  S  T  R  T  G  H

                                 KpnI
3721   TCATCACCATCACCATTGATAAGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGT
420     H  H  H  H  H
```

SEQ ID NO 15

pMPX-57 (β2 Adrenergic receptor (β2AR) cloned into pMPX-5 using PCR-introduced SalI and BamHI)

```
                                               Shine-Delgarno
2401   GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGGT

       SalI
2461   CGACATGGGGCAACCCGGGAACGGCAGCGCCTTCTTGCTGGCACCCAATGGAAGCCATGC
1          M  G  Q  P  G  N  G  S  A  F  L  L  A  P  N  G  S  H  A

2521   GCCGGACCACGACGTCACGCAGCAAAGGGACGAGGTGTGGGTGGTGGGCATGGGCATCGT
20      P  D  H  D  V  T  Q  Q  R  D  E  V  W  V  V  G  M  G  I  V

2581   CATGTCTCTCATCGTCCTGGCCATCGTGTTTGGCAATGTGCTGGTCATCACAGCCATTGC
40      M  S  L  I  V  L  A  I  V  F  G  N  V  L  V  I  T  A  I  A

2641   CAAGTTCGAGCGTCTGCAGACGGTCACCAACTACTTCATCACTTCACTGGCCTGTGCTGA
60      K  F  E  R  L  Q  T  V  T  N  Y  F  I  T  S  L  A  C  A  D

2701   TCTGGTCATGGGCCTAGCAGTGGTGCCCTTTGGGGCCGCCCATATTCTTATGAAAATGTG
80      L  V  M  G  L  A  V  V  P  F  G  A  A  H  I  L  M  K  M  W

2761   GACTTTTGGCAACTTCTGGTGCGAGTTTTGGACTTCCATTGATGTGCTGTGCGTCACGGC
100     T  F  G  N  F  W  C  E  F  W  T  S  I  D  V  L  C  V  T  A

2821   CAGCATTGAGACCCTGTGCGTGATCGCAGTGGATCGCTACTTTGCCATTACTTCACCTTT
120     S  I  E  T  L  C  V  I  A  V  D  R  Y  F  A  I  T  S  P  F
```



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2881   CAAGTACCAGAGCCTGCTGACCAAGAATAAGGCCCGGGTGATCATTCTGATGGTGTGGAT
140     K  Y  Q  S  L  L  T  K  N  K  A  R  V  I  I  L  M  V  W  I

2941   TGTGTCAGGCCTTAYCTCCTTCTTGCCCATTCAGATGCACTGGTACAGGGCCACCCACCA
160     V  S  G  L  X  S  F  L  P  I  Q  M  H  W  Y  R  A  T  H  Q

3001   GGAAGCCATCAACTGCTATGCCAATGAGACCTGCTGTGACTTCTTCACGAACCAAGCCTA
180     E  A  I  N  C  Y  A  N  E  T  C  C  D  F  F  T  N  Q  A  Y

3061   TGCCATTGCCTCTTCCATCGTGTCCTTCTACGTTCCCCTGGTGATCATGGTCTTCGTCTA
200     A  I  A  S  S  I  V  S  F  Y  V  P  L  V  I  M  V  F  V  Y

3121   CTCCAGGGTCTTTCAGGAGGCCAAAAGGCAGCTCCAGAAGATTGACAAATCTGAGGGCCG
220     S  R  V  F  Q  E  A  K  R  Q  L  Q  K  I  D  K  S  E  G  R

3181   CTTCCATGTCCAGAACCTTAGCCAGGTGGAGCAGGATGGGCGGACGGGGCATGGACTCCG
240     F  H  V  Q  N  L  S  Q  V  E  Q  D  G  R  T  G  H  G  L  R

3241   CAGATCTTCCAAGTTCTGCTTGAAGGAGCACAAAGCCCTCAAGACGTTAGGCATCATCAT
260     R  S  S  K  F  C  L  K  E  H  K  A  L  K  T  L  G  I  I  M

3301   GGGCACTTTCACCCTCTGCTGGCTGCCCTTCTTCATCGTTAACATTGTGCATGTGATCCA
280     G  T  F  T  L  C  W  L  P  F  F  I  V  N  I  V  H  V  I  Q

3361   GGATAACCTCATCCGTAAGGAAGTTTACATCCTCCTAAATTGGATAGGCTATGTCAATTC
300     D  N  L  I  R  K  E  V  Y  I  L  L  N  W  I  G  Y  V  N  S

3421   TGGTTTCAATCCCCTTATCTACTGCCGGAGCCCAGATTTCAGGATTGCCTTCCAGGAGCT
320     G  F  N  P  L  I  Y  C  R  S  P  D  F  R  I  A  F  Q  E  L

3481   TCTGTGCCTGCGCAGGTCTTCTTTGAAGGCCTATGGCAATGGCTACTCCAGCAACGGCAA
340     L  C  L  R  R  S  S  L  K  A  Y  G  N  G  Y  S  S  N  G  N

3541   CACAGGGGAGCAGAGTGGATATCACGTGGAACAGGAGAAAGAAAATAAACTGCTGTGTGA
360     T  G  E  Q  S  G  Y  H  V  E  Q  E  K  E  N  K  L  L  C  E

3601   AGACCTCCCAGGCACGGAAGACTTTGTGGGCCATCAAGGTACTGTGCCTAGCGATAACAT
380     D  L  P  G  T  E  D  F  V  G  H  Q  G  T  V  P  S  D  N  I

                                                            BamHI
3661   TGATTCACAAGGGAGGAATTGTAGTACAAATGACTCACTGCTATAATAAGGATCCCCGGG
400     D  S  Q  G  R  N  C  S  T  N  D  S  L  L
```

SEQ ID NO 16

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AATTGGTACC TCAATGATGA TGATGATGAT GCTTGCAGAG GACCCCATTC TG
```

SEQ ID NO 17

pMPX-1 (Human tumer necrosis factor receptor (TNFR-1) residues 41-455 cloned into pBAD-24 using PCR-introduced NcoI and XbaI)

```
                                                Shine-Delgarno
1261   TCGCAACTCTCTACTGTTTCTCCATACCCGTTTTTTTGGGCTAGCAGGAGGAATTCACCA
```



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          NcoI
1321      TGGATAGTGTGTGTCCCCAAGGAAAATATATCCACCCTCAAAATAATTCGATTTGCTGTA
1         M  D  S  V  C  P  Q  G  K  Y  I  H  P  Q  N  N  S  I  C  C

1381      CCAAGTGCCACAAAGGAACCTACTTGTACAATGACTGTCCAGGCCCGGGGCAGGATACGG
21        T  K  C  H  K  G  T  Y  L  Y  N  D  C  P  G  P  G  Q  D  T

1441      ACTGCAGGGAGTGTGAGAGCGGCTCCTTCACCGCTTCAGAAAACCACCTCAGACACTGCC
41        D  C  R  E  C  E  S  G  S  F  T  A  S  E  N  H  L  R  H  C

1501      TCAGCTGCTCCAAATGCCGAAAGGAAATGGGTCAGGTGGAGATCTCTTCTTGCACAGTGG
61        L  S  C  S  K  C  R  K  E  M  G  Q  V  E  I  S  S  C  T  V

1561      ACCGGGACACCGTGTGTGGCTGCAGGAAGAACCAGTACCGGCATTATTGGAGTGAAAACC
81        D  R  D  T  V  C  G  C  R  K  N  Q  Y  R  H  Y  W  S  E  N

1621      TTTTCCAGTGCTTCAATTGCAGCCTCTGCCTCAATGGGACCGTGCACCTCTCCTGCCAGG
101       L  F  Q  C  F  N  C  S  L  C  L  N  G  T  V  H  L  S  C  Q

1681      AGAAACAGAACACCGTGTGCACCTGCCATGCAGGTTTCTTTCTAAGAGAAAACGAGTGTG
121       E  K  Q  N  T  V  C  T  C  H  A  G  F  F  L  R  E  N  E  C

1741      TCTCCTGTAGTAACTGTAAGAAAAGCCTGGAGTGCACGAAGTTGTGCCTACCCCAGATTG
141       V  S  C  S  N  C  K  K  S  L  E  C  T  K  L  C  L  P  Q  I

1801      AGAATGTTAAGGGCACTGAGGACTCAGGCACCACAGTGCTGTTGCCCCTGGTCATTTTCT
161       E  N  V  K  G  T  E  D  S  G  T  T  V  L  L  P  L  V  I  F

1861      TTGGTCTTTGCCTTTTATCCCTCCTCTTCATTGGTTTAATGTATCGCTACCAACGGTGGA
181       F  G  L  C  L  L  S  L  L  F  I  G  L  M  Y  R  Y  Q  R  W

1921      AGTCCAAGCTCTACTCCATTGTTTGTGGGAAATCGACACCTGAAAAAGAGGGGGAGCTTG
201       K  S  K  L  Y  S  I  V  C  G  K  S  T  P  E  K  E  G  E  L

1981      AAGGAACTACTACTAAGCCCCTGGCCCCAAACCCAAGCTTCAGTCCCACTCCAGGCTTCA
221       E  G  T  T  T  K  P  L  A  P  N  P  S  F  S  P  T  P  G  F

2041      CCCCCACCCTGGGCTTCAGTCCCGTGCCCAGTTCCACCTTCACCTCCAGCTCCACCTATA
241       T  P  T  L  G  F  S  P  V  P  S  S  T  F  T  S  S  S  T  Y

2101      CCCCCGGTGACTGTCCCAACTTTGCGGCTCCCCGCAGAGAGGTGGCACCACCCTATCAGG
261       T  P  G  D  C  P  N  F  A  A  P  R  R  E  V  A  P  P  Y  Q

2161      GGGCTGACCCCATCCTTGCGACAGCCCTCGCCTCCGACCCCATCCCCAACCCCCTTCAGA
281       G  A  D  P  I  L  A  T  A  L  A  S  D  P  I  P  N  P  L  Q

2221      AGTGGGAGGACAGCGCCCACAAGCCACAGAGCCTAGACACTGATGACCCCGCGACGCTGT
301       K  W  E  D  S  A  H  K  P  Q  S  L  D  T  D  D  P  A  T  L

2281      ACGCCGTGGTGGAGAACGTGCCCCCGTTGCGCTGGAAGGAATTCGTGCGGCGCCTAGGGC
321       Y  A  V  V  E  N  V  P  P  L  R  W  K  E  F  V  R  R  L  G

2341      TGAGCGACCACGAGATCGATCGGCTGGAGCTGCAGAACGGGCGCTGCCTGCGCGAGGCGC
341       L  S  D  H  E  I  D  R  L  E  L  Q  N  G  R  C  L  R  E  A

2401      AATACAGCATGCTGGCGACCTGGAGGCGGCGCACGCCGCGGCGCGAGGCCACGCTGGAGC
361       Q  Y  S  M  L  A  T  W  R  R  R  T  P  R  R  E  A  T  L  E

2461      TGCTGGGACGCGTGCTCCGCGACATGGACCTGCTGGGCTGCCTGGAGGACATCGAGGAGG
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381         L  L  G  R  V  L  R  D  M  D  L  L  G  C  L  E  D  I  E  E

                                                                      XbaI
2521        CGCTTTGCGGCCCCGCCGCCCTCCCGCCCGCGCCCAGTCTTCTCAGATGATCTAGAGTCG
401         A  L  C  G  P  A  A  L  P  P  A  P  S  L  L  R
```

SEQ ID NO 18

pMPX-22 (Human tumer necrosis factor receptor (TNFR-1) residues 29-455 cloned into pMPX-18 using PCR-introduced SalI and KpnI)

```
                                    Shine-Delgarno          SalI
1621        CCATACCCGTTTTTTTGGGCTAGCAGGAGGAATTCACCCTGCAGGTCGACATGGGACTGG
1                                                                  M  G  L

1681        TCCCTCACCTAGGGGACAGGGAGAAGAGAGATAGTGTGTGTCCCCAAGGAAAATATATCC
4           V  P  H  L  G  D  R  E  K  R  D  S  V  C  P  Q  G  K  Y  I

1741        ACCCTCAAAATAATTCGATTTGCTGTACCAAGTGCCACAAAGGAACCTACTTGTACAATG
24          H  P  Q  N  N  S  I  C  C  T  K  C  H  K  G  T  Y  L  Y  N

1801        ACTGTCCAGGCCCGGGGCAGGATACGGACTGCAGGGAGTGTGAGAGCGGCTCCTTCACCG
44          D  C  P  G  P  G  Q  D  T  D  C  R  E  C  E  S  G  S  F  T

1861        CTTCAGAAAACCACCTCAGACACTGCCTCAGCTGCTCCAAATGCCGAAAGGAAATGGGTC
64          A  S  E  N  H  L  R  H  C  L  S  C  S  K  C  R  K  E  M  G

1921        AGGTGGAGATCTCTTCTTGCACAGTGGACCGGGACACCGTGTGTGGCTGCAGGAAGAACC
84          Q  V  E  I  S  S  C  T  V  D  R  D  T  V  C  G  C  R  K  N

1981        AGTACCGGCATTATTGGAGTGAAAACCTTTTCCAGTGCTTCAATTGCAGCCTCTGCCTCA
104         Q  Y  R  H  Y  W  S  E  N  L  F  Q  C  F  N  C  S  L  C  L

2041        ATGGGACCGTGCACCTCTCCTGCCAGGAGAAACAGAACACCGTGTGCACCTGCCATGCAG
124         N  G  T  V  H  L  S  C  Q  E  K  Q  N  T  V  C  T  C  H  A

2101        GTTTCTTTCTAAGAGAAAACGAGTGTGTCTCCTGTAGTAACTGTAAGAAAAGCCTGGAGT
144         G  F  F  L  R  E  N  E  C  V  S  C  S  N  C  K  K  S  L  E

2161        GCACGAAGTTGTGCCTACCCCAGATTGAGAATGTTAAGGGCACTGAGGACTCAGGCACCA
164         C  T  K  L  C  L  P  Q  I  E  N  V  K  G  T  E  D  S  G  T

2221        CAGTGCTGTTGCCCCTGGTCATTTTCTTTGGTCTTTGCCTTTTATCCCTCCTCTTCATTG
184         T  V  L  L  P  L  V  I  F  F  G  L  C  L  L  S  L  L  F  I

2281        GTTTAATGTATCGCTACCAACGGTGGAAGTCCAAGCTCTACTCCATTGTTTGTGGGAAAT
204         G  L  M  Y  R  Y  Q  R  W  K  S  K  L  Y  S  I  V  C  G  K

2341        CGACACCTGAAAAAGAGGGGGAGCTTGAAGGAACTACTACTAAGCCCCTGGCCCCAAACC
224         S  T  P  E  K  E  G  E  L  E  G  T  T  T  K  P  L  A  P  N
```

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2401    CAAGCTTCAGTCCCACTCCAGGCTTCACCCCCACCCTGGGCTTCAGTCCCGTGCCCAGTT
244     P  S  F  S  P  T  P  G  F  T  P  T  L  G  F  S  P  V  P  S

2461    CCACCTTCACCTCCAGCTCCACCTATACCCCCGGTGACTGTCCCAACTTTGCGGCTCCCC
264     S  T  F  T  S  S  S  T  Y  T  P  G  D  C  P  N  F  A  A  P

2521    GCAGAGAGGTGGCACCACCCTATCAGGGGGCTGACCCCATCCTTGCGACAGCCCTCGCCT
284     R  R  E  V  A  P  P  Y  Q  G  A  D  P  I  L  A  T  A  L  A

2581    CCGACCCCATCCCCAACCCCCTTCAGAAGTGGGAGGACAGCGCCCACAAGCCACAGAGCC
304     S  D  P  I  P  N  P  L  Q  K  W  E  D  S  A  H  K  P  Q  S

2641    TAGACACTGATGACCCCGCGACGCTGTACGCCGTGGTGGAGAACGTGCCCCCGTTGCGCT
324     L  D  T  D  D  P  A  T  L  Y  A  V  V  E  N  V  P  P  L  R

2701    GGAAGGAATTCGTGCGGCGCCTAGGGCTGAGCGACCACGAGATCGATCGGCTGGAGCTGC
344     W  K  E  F  V  R  R  L  G  L  S  D  H  E  I  D  R  L  E  L

2761    AGAACGGGCGCTGCCTGCGCGAGGCGCAATACAGCATGCTGGCGACCTGGAGGCGGCGCA
364     Q  N  G  R  C  L  R  E  A  Q  Y  S  M  L  A  T  W  R  R  R

2821    CGCCGCGGCGCGAGGCCACGCTGGAGCTGCTGGGACGCGTGCTCCGCGACATGGACCTGC
384     T  P  R  R  E  A  T  L  E  L  L  G  R  V  L  R  D  M  D  L

2881    TGGGCTGCCTGGAGGACATCGAGGAGGCGCTTTGCGGCCCCGCCGCCCTCCCGCCCGCGC
404     L  G  C  L  E  D  I  E  E  A  L  C  G  P  A  A  L  P  P  A

                                    KpnI
2941    CCAGTCTTCTCAGATAATAAGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTT
424     P  S  L  L  R
```

SEQ ID NO 19

pMPX-40 (Human tumer necrosis factor (TNF) cloned into pMPX-6 using PCR-introduced EcoRI and HindIII)

```
                                     EcoRI
                         Shine-Delgarno
2581    GTTTTTTTGGGCTAGCAGGAGGAATTCATGAGCACTGAAAGCATGATCCGGGACGTGGAG
1                                          M  S  T  E  S  M  I  R  D  V  E

2641    CTGGCCGAGGAGGCGCTCCCCAAGAAGACAGGGGGGCCCCAGGGCTCCAGGCGGTGCTTG
12       L  A  E  E  A  L  P  K  K  T  G  G  P  Q  G  S  R  R  C  L

2701    TTCCTCAGCCTCTTCTCCTTCCTGATCGTGGCAGGCGCCACCACGCTCTTCTGCCTGCTG
32       F  L  S  L  F  S  F  L  I  V  A  G  A  T  T  L  F  C  L  L

2761    CACTTTGGAGTGATCGGCCCCCAGAGGGAAGAGTTCCCCAGGGACCTCTCTCTAATCAGC
52       H  F  G  V  I  G  P  Q  R  E  E  F  P  R  D  L  S  L  I  S

2821    CCTCTGGCCCAGGCAGTCAGATCATCTTCTCGAACCCCGAGTGACAAGCCTGTAGCCCAT
72       P  L  A  Q  A  V  R  S  S  S  R  T  P  S  D  K  P  V  A  H

2881    GTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTGAACCGCCGGGCCAATGCC
92       V  V  A  N  P  Q  A  E  G  Q  L  Q  W  L  N  R  R  A  N  A
```

```
2941    CTCCTGGCCAATGGCGTGGAGCTGAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTG
112      L  L  A  N  G  V  E  L  R  D  N  Q  L  V  V  P  S  E  G  L

3001    TACCTCATCTACTCCCAGGTCCTCTTCAAGGGCCAAGGCTGCCCCTCCACCCATGTGCTC
132      Y  L  I  Y  S  Q  V  L  F  K  G  Q  G  C  P  S  T  H  V  L

3061    CTCACCCACACCATCAGCCGCATCGCCGTCTCCTACCAGACCAAGGTCAACCTCCTCTCT
152      L  T  H  T  I  S  R  I  A  V  S  Y  Q  T  K  V  N  L  L  S

3121    GCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTGAGGCCAAGCCCTGGTAT
172      A  I  K  S  P  C  Q  R  E  T  P  E  G  A  E  A  K  P  W  Y

3181    GAGCCCATCTATCTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCTGAG
192      E  P  I  Y  L  G  G  V  F  Q  L  E  K  G  D  R  L  S  A  E

3241    ATCAATCGGCCCGACTATCTCGACTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATT
212      I  N  R  P  D  Y  L  D  F  A  E  S  G  Q  V  Y  F  G  I  I

                    HindIII
3301    GCCCTGTGATAAGCTTGGCCCGCGGGCCCGGGATCCACCGGATCTAGATAACTGATCATA
232      A  L
```

SEQ ID NO 20

pMPX-52 (*toxR*-EGF cloned into pMPX-6 using PCR-introduced KpnI and HindIII)

```
                          Shine-Delgarno          KpnI
2581    GTTTTTTTGGGCTAGCAGGAGGAATTCACCATGGTACCATGAACTTGGGGAATCGACTGT
1                                                M  N  L  G  N  R  L

2641    TTATTCTGATAGCGGTCTTACTTCCCCTCGCAGTATTACTGCTCAATAGTGACTCTGAAT
8       F  I  L  I  A  V  L  L  P  L  A  V  L  L  L  N  S  D  S  E

2701    GTCCCCTGTCCCACGATGGGTACTGCCTCCATGATGGTGTGTGCATGTATATTGAAGCAT
28      C  P  L  S  H  D  G  Y  C  L  H  D  G  V  C  M  Y  I  E  A

2761    TGGACAAGTATGCATGCAACTGTGTTGTTGGCTACATCGGGGAGCGATGTCAGTACCGAG
48      L  D  K  Y  A  C  N  C  V  V  G  Y  I  G  E  R  C  Q  Y  R

                                       HindIII
2821    ACCTGAAGTGGTGGGAACTGCGCTAATAAGCTTGGCCCGCGGGCCCGGGATCCACCGGAT
          68   D L K W W E L R
```

Non-bold, underlined sequence is *toxR* transmembrane domain segment that constitutes *toxR* residues 178-198. The remaining sequence is from human EGF constituting EGF residues 971-1023.

SEQ ID NO 21

pMPX-27 (*toxR*-invasin cloned into pMPX-6 using PCR-introduced EcoRI and PstI)

EcoRI
Shine-Delgarno

```
2581   GTTTTTTTGGGCTAGCAGGAGGAATTCACCATGAACTTGGGGAATCGACTGTTTATTCTG
1                                      M  N  L  G  N  R  L  F  I  L

2641   ATAGCGGTCTTACTTCCCCTCGCAGTATTACTGCTCTCATTCACATTGAGCGTCACCGTT
11      I  A  V  L  L  P  L  A  V  L  L  L  S  F  T  L  S  V  T  V

2701   CAGCAGCCTCAGTTGACATTAACGGCGGCCGTCATTGGTGATGGCGCACCGGCTAATGGG
31      Q  Q  P  Q  L  T  L  T  A  A  V  I  G  D  G  A  P  A  N  G

2761   AAAACTGCAATCACCGTTGAGTTCACCGTTGCTGATTTTGAGGGGAAACCCTTAGCCGGG
51      K  T  A  I  T  V  E  F  T  V  A  D  F  E  G  K  P  L  A  G

2821   CAGGAGGTGGTGATAACCACCAATAATGGTGCGCTACCGAATAAAATCACGGAAAAGACA
71      Q  E  V  V  I  T  T  N  N  G  A  L  P  N  K  I  T  E  K  T

2881   GATGCAAATGGCGTCGCGCGCATTGCATTAACCAATACGACAGATGGCGTGACGGTAGTC
91      D  A  N  G  V  A  R  I  A  L  T  N  T  T  D  G  V  T  V  V

2941   ACAGCAGAAGTGGAGGGGCAACGGCAAAGTGTTGATACCCACTTTGTTAAGGGTACTATC
111     T  A  E  V  E  G  Q  R  Q  S  V  D  T  H  F  V  K  G  T  I

3001   GCGGCGGATAAATCCACTCTGGCTGCGGTACCGACATCTATCATCGCTGATGGTCTAATG
131     A  A  D  K  S  T  L  A  A  V  P  T  S  I  I  A  D  G  L  M

3061   GCTTCAACCATCACGTTGGAGTTGAAGGATACCTATGGGGACCCGCAGGCTGGCGCGAAT
151     A  S  T  I  T  L  E  L  K  D  T  Y  G  D  P  Q  A  G  A  N

3121   GTGGCTTTTGACACAACCTTAGGCAATATGGGCGTTATCACGGATCACAATGACGGCACT
171     V  A  F  D  T  T  L  G  N  M  G  V  I  T  D  H  N  D  G  T

3181   TATAGCGCACCATTGACCAGTACCACGTTGGGGGTAGCAACAGTAACGGTGAAAGTGGAT
191     Y  S  A  P  L  T  S  T  T  L  G  V  A  T  V  T  V  K  V  D

3241   GGGGCTGCGTTCAGTGTGCCGAGTGTGACGGTTAATTTCACGGCAGATCCTATTCCAGAT
211     G  A  A  F  S  V  P  S  V  T  V  N  F  T  A  D  P  I  P  D

3301   GCTGGCCGCTCCAGTTTCACCGTCTCCACACCGGATATCTTGGCTGATGGCACGATGAGT
231     A  G  R  S  S  F  T  V  S  T  P  D  I  L  A  D  G  T  M  S

3361   TCCACATTATCCTTTGTCCCTGTCGATAAGAATGGCCATTTTATCAGTGGGATGCAGGGC
251     S  T  L  S  F  V  P  V  D  K  N  G  H  F  I  S  G  M  Q  G

3421   TTGAGTTTTACTCAAAACGGTGTGCCGGTGAGTATTAGCCCCATTACCGAGCAGCCAGAT
271     L  S  F  T  Q  N  G  V  P  V  S  I  S  P  I  T  E  Q  P  D

3481   AGCTATACCGCGACGGTGGTTGGGAATAGTGTCGGTGATGTCACAATCACGCCGCAGGTT
291     S  Y  T  A  T  V  V  G  N  S  V  G  D  V  T  I  T  P  Q  V

3541   GATACCCTGATACTGAGTACATTGCAGAAAAAAATATCCCTATTCCCGGTACCTACGCTG
311     D  T  L  I  L  S  T  L  Q  K  K  I  S  L  F  P  V  P  T  L

3601   ACCGGTATTCTGGTTAACGGGCAAAATTTCGCTACGGATAAAGGGTTCCCGAAAACGATC
331     T  G  I  L  V  N  G  Q  N  F  A  T  D  K  G  F  P  K  T  I
```

```
3661   TTTAAAAACGCCACATTCCAGTTACAGATGGATAACGATGTTGCTAATAATACTCAGTAT
351     F  K  N  A  T  F  Q  L  Q  M  D  N  D  V  A  N  N  T  Q  Y

3721   GAGTGGTCGTCGTCATTCACACCCAATGTATCGGTTAACGATCAGGGTCAGGTGACGATT
371     E  W  S  S  S  F  T  P  N  V  S  V  N  D  Q  G  Q  V  T  I

3781   ACCTACCAAACCTATAGCGAAGTGGCTGTGACGGCGAAAAGTAAAAAATTCCCAAGTTAT
391     T  Y  Q  T  Y  S  E  V  A  V  T  A  K  S  K  K  F  P  S  Y

3841   TCGGTGAGTTATCGGTTCTACCCAAATCGGTGGATATACGATGGCGGCAGATCGCTGGTA
411     S  V  S  Y  R  F  Y  P  N  R  W  I  Y  D  G  G  R  S  L  V

3901   TCCAGTCTCGAGGCCAGCAGACAATGCCAAGGTTCAGATATGTCTGCGGTTCTTGAATCC
431     S  S  L  E  A  S  R  Q  C  Q  G  S  D  M  S  A  V  L  E  S

3961   TCACGTGCAACCAACGGAACGCGTGCGCCTGACGGGACATTGTGGGGCGAGTGGGGGAGC
451     S  R  A  T  N  G  T  R  A  P  D  G  T  L  W  G  E  W  G  S

4021   TTGACCGCGTATAGTTCTGATTGGCAATCTGGTGAATATTGGGTCAAAAAGACCAGCACG
471     L  T  A  Y  S  S  D  W  Q  S  G  E  Y  W  V  K  K  T  S  T

4081   GATTTTGAAACCATGAATATGGACACAGGCGCACTGCAACCAGGGCCTGCATACTTGGCG
491     D  F  E  T  M  N  M  D  T  G  A  L  Q  P  G  P  A  Y  L  A

                                          PstI
4141   TTCCCGCTCTGTGCGCTGTCAATATAACTGCAGGCATGCAAGCTTGGCCCGCGGGCCCGG
511     F  P  L  C  A  L  S  I
```

Non-bold, underlined sequence is *toxR* transmembrane domain segment that constitutes *toxR* residues 178-198. The remaining sequence is from *Yersinia pseudotuberculosis* invasin constituting *inv* residues 490-986.

SEQ ID NO 22

pMPX-59 (*phoA* leader cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                              Shine-Delgarno        PstI
2401   GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
                                                                     M

2461   GTCACGGCCGAGACTTATAGTCGCTTTGTTTTTATTTTTTAATGTATTTGTACATGGAGA
2       S  R  P  R  L  I  V  A  L  F  L  F  F  N  V  F  V  H  G  E

2521   AAATAAAGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGTTACTGTTTACCCCTGT
22      N  K  V  K  Q  S  T  I  A  L  A  L  L  P  L  L  F  T  P  V

                                XbaI
2581   GACAAAAGCCCGGACACCAGAATCTAGA
42      T  K  A  R  T  P  E  S  R
```

PhoA leader (residues 1-48) from *E. coli* MG1655 cloned into pMPX-5. Create chimeric fusions with the *phoA* leader by cloning into XbaI and introducing a stop sequence.

SEQ ID NO 23

pMPX-60 (complete *phoA* cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                                                   Shine-Delgarno      PstI
2401    GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
1                                                                  M

2461    GTCACGGCCGAGACTTATAGTCGCTTTGTTTTTATTTTTTAATGTATTTGTACATGGAGA
2         S  R  P  R  L  I  V  A  L  F  L  F  F  N  V  F  V  H  G  E

2521    AAATAAAGTGAAACAAAGCACTATTGCACTGGCACTCTTACCGTTACTGTTTACCCCTGT
22        N  K  V  K  Q  S  T  I  A  L  A  L  L  P  L  L  F  T  P  V

2581    GACAAAAGCCCGGACACCAGAAATGCCTGTTCTGGAAAACCGGGCTGCTCAGGGCGATAT
42        T  K  A  R  T  P  E  M  P  V  L  E  N  R  A  A  Q  G  D  I

2641    TACTGCACCCGGCGGTGCTCGCCGTTTAACGGGTGATCAGACTGCCGCTCTGCGTGATTC
62        T  A  P  G  G  A  R  R  L  T  G  D  Q  T  A  A  L  R  D  S

2701    TCTTAGCGATAAACCTGCAAAAAATATTATTTTGCTGATTGGCGATGGGATGGGGGACTC
82        L  S  D  K  P  A  K  N  I  I  L  L  I  G  D  G  M  G  D  S

2761    GGAAATTACTGCCGCACGTAATTATGCCGAAGGTGCGGGCGGCTTTTTTAAAGGTATAGA
102       E  I  T  A  A  R  N  Y  A  E  G  A  G  G  F  F  K  G  I  D

2821    TGCCTTACCGCTTACCGGGCAATACACTCACTATGCGCTGAATAAAAAAACCGGCAAACC
122       A  L  P  L  T  G  Q  Y  T  H  Y  A  L  N  K  K  T  G  K  P

2881    GGACTACGTCACCGACTCGGCTGCATCAGCAACCGCCTGGTCAACCGGTGTCAAAACCTA
142       D  Y  V  T  D  S  A  A  S  A  T  A  W  S  T  G  V  K  T  Y

2941    TAACGGCGCGCTGGGCGTCGATATTCACGAAAAAGATCACCCAACGATTCTGGAAATGGC
162       N  G  A  L  G  V  D  I  H  E  K  D  H  P  T  I  L  E  M  A

3001    AAAAGCCGCAGGTCTGGCGACCGGTAACGTTTCTACCGCAGAGTTGCAGGATGCCACGCC
182       K  A  A  G  L  A  T  G  N  V  S  T  A  E  L  Q  D  A  T  P

3061    CGCTGCGCTGGTGGCACATGTGACCTCGCGCAAATGCTACGGTCCGAGCGCGACCAGTGA
202       A  A  L  V  A  H  V  T  S  R  K  C  Y  G  P  S  A  T  S  E

3121    AAAATGTCCGGGTAACGCTCTGGAAAAAGGCGGAAAAGGATCGATTACCGAACAGCTGCT
222       K  C  P  G  N  A  L  E  K  G  G  K  G  S  I  T  E  Q  L  L

3181    TAACGCTCGTGCCGACGTTACGCTTGGCGGCGGCGCAAAAACCTTTGCTGAAACGGCAAC
242       N  A  R  A  D  V  T  L  G  G  G  A  K  T  F  A  E  T  A  T

3241    CGCTGGTGAATGGCAGGGAAAAACGCTGCGTGAACAGGCACAGGCGCGTGGTTATCAGTT
262       A  G  E  W  Q  G  K  T  L  R  E  Q  A  Q  A  R  G  Y  Q  L

3301    GGTGAGCGATGCTGCCTCACTGAATTCGGTGACGGAAGCGAATCAGCAAAAACCCCTGCT
282       V  S  D  A  A  S  L  N  S  V  T  E  A  N  Q  Q  K  P  L  L

3361    TGGCCTGTTTGCTGACGGCAATATGCCAGTGCGCTGGCTAGGACCGAAAGCAACGTACCA
302       G  L  F  A  D  G  N  M  P  V  R  W  L  G  P  K  A  T  Y  H

3421    TGGCAATATCGATAAGCCCGCAGTCACCTGTACGCCAAATCCGCAACGTAATGACAGTGT
322       G  N  I  D  K  P  A  V  T  C  T  P  N  P  Q  R  N  D  S  V

3481    ACCAACCCTGGCGCAGATGACCGACAAAGCCATTGAATTGTTGAGTAAAAATGAGAAAGG
```



```
342       P  T  L  A  Q  M  T  D  K  A  I  E  L  L  S  K  N  E  K  G

3541     CTTTTTCCTGCAAGTTGAAGGTGCGTCAATCGATAAACAGGATCATGCTGCGAATCCTTG
362       F  F  L  Q  V  E  G  A  S  I  D  K  Q  D  H  A  A  N  P  C

3601     TGGGCAAATTGGCGAGACGGTCGATCTCGATGAAGCCGTACAACGGGCGCTGGAATTCGC
382       G  Q  I  G  E  T  V  D  L  D  E  A  V  Q  R  A  L  E  F  A

3661     TAAAAAGGAGGGTAACACGCTGGTCATAGTCACCGCTGATCACGCCCACGCCAGCCAGAT
402       K  K  E  G  N  T  L  V  I  V  T  A  D  H  A  H  A  S  Q  I

3721     TGTTGCGCCGGATACCAAAGCTCCGGGCCTCACCCAGGCGCTAAATACCAAAGATGGCGC
422       V  A  P  D  T  K  A  P  G  L  T  Q  A  L  N  T  K  D  G  A

3781     AGTGATGGTGATGAGTTACGGGAACTCCGAAGAGGATTCACAAGAACATACCGGCAGTCA
442       V  M  V  M  S  Y  G  N  S  E  E  D  S  Q  E  H  T  G  S  Q

3841     GTTGCGTATTGCGGCGTATGGCCCGCATGCCGCCAATGTTGTTGGACTGACCGACCAGAC
462       L  R  I  A  A  Y  G  P  H  A  A  N  V  V  G  L  T  D  Q  T

                                                 XbaI
3901     CGATCTCTTCTACACCATGAAAGCCGCTCTGGGGCTGAAATCTAGA
482       D  L  F  Y  T  M  K  A  A  L  G  L  K  S  R
```

Complete PhoA from *E. coli* MG1655 cloned into pMPX-5. Create chimeric fusions with the *phoA* by cloning into XbaI and introducing a stop sequence.

SEQ ID NO 24

pMPX-62 (MalE residues 1-28 cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                               Shine-Delgarno      PstI
2401     GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
1                                                                    M

2461     GAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAACGACGATGATGTTTTC
2         K  I  K  T  G  A  R  I  L  A  L  S  A  L  T  T  M  M  F  S

                             XbaI
2521     CGCCTCGGCTCTCGCCAAAATCTCTAGA
22        A  S  A  L  A  K  I  S  R
```

MalE residues 1-28 from *E. coli* MG1655 cloned into pMPX-5. Create chimeric fusions with the *malE* by cloning into XbaI and introducing a stop sequence.

SEQ ID NO 25

pMPX-61 (MalE residues 1-370 cloned into pMPX-5 using PCR-introduced PstI and XbaI)

```
                                               Shine-Delgarno      PstI
2401     GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTCAGCAGGATCACATTCTGCAGAT
```

```
1                                                                    M

2461   GAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAACGACGATGATGTTTTC
2       K  I  K  T  G  A  R  I  L  A  L  S  A  L  T  T  M  M  F  S

2521   CGCCTCGGCTCTCGCCAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAA
22      A  S  A  L  A  K  I  E  E  G  K  L  V  I  W  I  N  G  D  K

2581   AGGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAAGT
42      G  Y  N  G  L  A  E  V  G  K  K  F  E  K  D  T  G  I  K  V

2641   CACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTCCCACAGGTTGCGGCAACTGGCGA
62      T  V  E  H  P  D  K  L  E  E  K  F  P  Q  V  A  A  T  G  D

2701   TGGCCCTGACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGCCT
82      G  P  D  I  I  F  W  A  H  D  R  F  G  G  Y  A  Q  S  G  L

2761   GTTGGCTGAAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGGGA
102     L  A  E  I  T  P  D  K  A  F  Q  D  K  L  Y  P  F  T  W  D

2821   TGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCT
122     A  V  R  Y  N  G  K  L  I  A  Y  P  I  A  V  E  A  L  S  L

2881   GATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCGCT
142     I  Y  N  K  D  L  L  P  N  P  P  K  T  W  E  E  I  P  A  L

2941   GGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCGTA
162     D  K  E  L  K  A  K  G  K  S  A  L  M  F  N  L  Q  E  P  Y

3001   CTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGCAA
182     F  T  W  P  L  I  A  A  D  G  G  Y  A  F  K  Y  E  N  G  K

3061   GTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTCCT
202     Y  D  I  K  D  V  G  V  D  N  A  G  A  K  A  G  L  T  F  L

3121   GGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCAGAAGC
222     V  D  L  I  K  N  K  H  M  N  A  D  T  D  Y  S  I  A  E  A

3181   TGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAACAT
242     A  F  N  K  G  E  T  A  M  T  I  N  G  P  W  A  W  S  N  I

3241   CGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAGGGTCAACCATC
262     D  T  S  K  V  N  Y  G  V  T  V  L  P  T  F  K  G  Q  P  S

3301   CAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCGAACAAAGAGCT
282     K  P  F  V  G  V  L  S  A  G  I  N  A  A  S  P  N  K  E  L

3361   GGCGAAAGAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGGAAGCGGTTAATAA
302     A  K  E  F  L  E  N  Y  L  L  T  D  E  G  L  E  A  V  N  K

3421   AGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGAGTTGGCGAAAGATCC
322     D  K  P  L  G  A  V  A  L  K  S  Y  E  E  E  L  A  K  D  P

3481   ACGTATTGCCGCCACCATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACATCCCGCA
342     R  I  A  A  T  M  E  N  A  Q  K  G  E  I  M  P  N  I  P  Q

                                             XbaI
3541   GATGTCCGCTTTCTGGTATGCCGTGCGTTCTAGA
362     M  S  A  F  W  Y  A  V  R  S  R
```

MalE residues 1-370 from *E. coli* MG1655 cloned into pMPX-5. Create chimeric fusions with the *malE* by cloning into XbaI and introducing a stop sequence.

SEQ ID NO 26

pMPX-17 (complete *tig* and groESL, both with complete native control region cloned into pMPX-5 using PCR-introduced NarI and HindIII. The *tig* and *groESL* regions are joined using XbaI). Construct to be used on same vector as protein to be expressed or as a template for insertion into pACYC184.

NarI
181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCA**GGCGCC**

241 *ATACGCGACA GCGCGCAATA ACCGTTCTCG ACTCATAAAA GTGATGCCGC TATAATGCCG*
301 *CGTCCTATTT GAATGCTTTC GGGATGATTC TGGTAACAGG GAATGTGATT GATTATAAGA*
361 *ACATCCCGGT TCCGCGAAGC CAACAACCTG TGCTTGCGGG GTAAGAGTTG ACCGAGCACT*

+1 *tig*
421 *GTGATTTTTT GAGGTAACAA* G**ATG**CAAGTT TCAGTTGAAA CCACTCAAGG CCTTGGCCGC
→

481 CGTGTAACGA TTACTATCGC TGCTGACAGC ATCGAGACCG CTGTTAAAAG CGAGCTGGTC
541 AACGTTGCGA AAAAAGTACG TATTGACGGC TTCCGCAAAG GCAAAGTGCC AATGAATATC
601 GTTGCTCAGC GTTATGGCGC GTCTGTACGC CAGGACGTTC TGGGTGACCT GATGAGCCGT
661 AACTTCATTG ACGCCATCAT TAAAGAAAAA ATCAATCCGG CTGGCGCACC GACTTATGTT
721 CCGGGCGAAT ACAAGCTGGG TGAAGACTTC ACTTACTCTG TAGAGTTTGA AGTTTATCCG
781 GAAGTTGAAC TGCAGGGTCT GGAAGCGATC GAAGTTGAAA AACCGATCGT TGAAGTGACC
841 GACGCTGACG TTGACGGCAT GCTGGATACT CTGCGTAAAC AGCAGGCGAC CTGGAAAGAA
901 AAAGACGGCG CTGTTGAAGC AGAAGACCGC GTAACCATCG ACTTCACCGG TTCTGTAGAC
961 GGCGAAGAGT TCGAAGGCGG TAAAGCGTCT GATTTCGTAC TGGCGATGGG CCAGGGTCGT
1021 ATGATCCCGG GCTTTGAAGA CGGTATCAAA GGCCACAAAG CTGGCGAAGA GTTCACCATC
1081 GACGTGACCT TCCCGGAAGA ATACCACGCA GAAAACCTGA AAGGTAAAGC AGCGAAATTC
1141 GCTATCAACC TGAAGAAAGT TGAAGAGCGT GAACTGCCGG AACTGACTGC AGAATTCATC
1201 AAACGTTTCG GCGTTGAAGA TGGTTCCGTA GAAGGTCTGC GCGCTGAAGT GCGTAAAAAC
1261 ATGGAGCGCG AGCTGAAGAG CGCCATCCGT AACCGCGTTA AGTCTCAGGC GATCGAAGGT

1321 CTGGTAAAAG CTAACGACAT CGACGTACCG GCTGCGCTGA TCGACAGCGA
AATCGACGTT
1381 CTGCGTCGCC AGGCTGCACA GCGTTTCGGT GGCAACGAAA AACAAGCTCT
GGAACTGCCG
1441 CGCGAACTGT TCGAAGAACA GGCTAAACGC CGCGTAGTTG TTGGCCTGCT
GCTGGGCGAA
1501 GTTATCCGCA CCAACGAGCT GAAAGCTGAC GAAGAGCGCG TGAAAGGCCT
GATCGAAGAG
1561 ATGGCTTCTG CGTACGAAGA TCCGAAAGAA GTTATCGAGT TCTACAGCAA
AAACAAAGAA
1621 CTGATGGACA ACATGCGCAA TGTTGCTCTG GAAGAACAGG CTGTTGAAGC
TGTACTGGCG

Stop

tig
1681 AAAGCGAAAG TGACTGAAAA AGAAACCACT TTCAACGAGC TGATGAACCA
GCAGGCG**TAA**

1741 TAATAATCTA GAGGTAGCAC AATCAGATTC GCTTATGACG GCGATGAAGA
AATTGCGATG
1801 AAATGTGAGG TGAATCAGGG TTTTCACCCG ATTTTGTGCT GATCAGAATT
TTTTTTCTTT
1861 TTCCCCCTTG AAGGGGCGAA GCCTCATCCC CATTTCTCTG GTCACCAGCC
GGGAAACCAC

+1

groES
1921 GTAAGCTCCG GCGTCACCCA TAACAGATAC GGACTTTCTC AAAGGAGAGT
TATCA**ATG**AA

→

1981 TATTCGTCCA TTGCATGATC GCGTGATCGT CAAGCGTAAA GAAGTTGAAA
CTAAATCTGC
2041 TGGCGGCATC GTTCTGACCG GCTCTGCAGC GGCTAAATCC ACCCGCGGCG
AAGTGCTGGC
2101 TGTCGGCAAT GGCCGTATCC TTGAAAATGG CGAAGTGAAG CCGCTGGATG
TGAAAGTTGG
2161 CGACATCGTT ATTTTCAACG ATGGCTACGG TGTGAAATCT GAGAAGATCG
ACAATGAAGA

Stop *groES*
2221 AGTGTTGATC ATGTCCGAAA GCGACATTCT GGCAATTGTT GAAGCG**TAA**T
CCGCGCACGA

+1 *groEL*
2281 CACTGAACAT ACGAATTTAA GGAATAAAGA TA**ATG**GCAGC TAAAGACGTA
AAATTCGGTA

→

2341 ACGACGCTCG TGTGAAAATG CTGCGCGGCG TAAACGTACT GGCAGATGCA
GTGAAAGTTA
2401 CCCTCGGTCC AAAAGGCCGT AACGTAGTTC TGGATAAATC TTTCGGTGCA
CCGACCATCA
2461 CCAAAGATGG TGTTTCCGTT GCTCGTGAAA TCGAACTGGA AGACAAGTTC
GAAAATATGG
2521 GTGCGCAGAT GGTGAAAGAA GTTGCCTCTA AAGCAAACGA CGCTGCAGGC
GACGGTACCA
2581 CCACTGCAAC CGTACTGGCT CAGGCTATCA TCACTGAAGG TCTGAAAGCT
GTTGCTGCGG

```
2641    GCATGAACCC GATGGACCTG AAACGTGGTA TCGACAAAGC GGTTACCGCT
GCAGTTGAAG
2701    AACTGAAAGC GCTGTCCGTA CCATGCTCTG ACTCTAAAGC GATTGCTCAG
GTTGGTACCA
2761    TCTCCGCTAA CTCCGACGAA ACCGTAGGTA AACTGATCGC TGAAGCGATG
GACAAAGTCG
2821    GTAAAGAAGG CGTTATCACC GTTGAAGACG GTACCGGTCT GCAGGACGAA
CTGGACGTGG
2881    TTGAAGGTAT GCAGTTCGAC CGTGGCTACC TGTCTCCTTA CTTCATCAAC
AAGCCGGAAA
2941    CTGGCGCAGT AGAACTGGAA AGCCCGTTCA TCCTGCTGGC TGACAAGAAA
ATCTCCAACA
3001    TCCGCGAAAT GCTGCCGGTT CTGGAAGCTG TTGCCAAAGC AGGCAAACCG
CTGCTGATCA
3061    TCGCTGAAGA TGTAGAAGGC GAAGCGCTGG CAACTCTGGT TGTTAACACC
ATGCGTGGCA
3121    TCGTGAAAGT CGCTGCGGTT AAAGCACCGG GCTTCGGCGA TCGTCGTAAA
GCTATGCTGC
3181    AGGATATCGC AACCCTGACT GGCGGTACCG TGATCTCTGA AGAGATCGGT
ATGGAGCTGG
3241    AAAAAGCAAC CCTGGAAGAC CTGGGTCAGG CTAAACGTGT TGTGATCAAC
AAAGACACCA
3301    CCACTATCAT CGATGGCGTG GGTGAAGAAG CTGCAATCCA GGGCCGTGTT
GCTCAGATCC
3361    GTCAGCAGAT TGAAGAAGCA ACTTCTGACT ACGACCGTGA AAAACTGCAG
GAACGCGTAG
3421    CGAAACTGGC AGGCGGCGTT GCAGTTATCA AAGTGGGTGC TGCTACCGAA
GTTGAAATGA
3481    AAGAGAAAAA AGCACGCGTT GAAGATGCCC TGCACGCGAC CCGTGCTGCG
GTAGAAGAAG
3541    GCGTGGTTGC TGGTGGTGGT GTTGCGCTGA TCCGCGTAGC GTCTAAACTG
GCTGACCTGC
3601    GTGGTCAGAA CGAAGACCAG AACGTGGGTA TCAAAGTTGC ACTGCGTGCA
ATGGAAGCTC
3661    CGCTGCGTCA GATCGTATTG AACTGCGGCG AAGAACCGTC TGTTGTTGCT
AACACCGTTA
3721    AAGGCGGCGA CGGCAACTAC GGTTACAACG CAGCAACCGA AGAATACGGC
AACATGATCG
3781    ACATGGGTAT CCTGGATCCA ACCAAAGTAA CTCGTTCTGC TCTGCAGTAC
GCAGCTTCTG
3841    TGGCTGGCCT GATGATCACC ACCGAATGCA TGGTTACCGA CCTGCCGAAA
AACGATGCAG
```

Stop

groEL

3901 CTGACTTAGG CGCTGCTGGC GGTATGGGCG GCATGGGTGG CATGGGCGGC
ATGATG**<u>TAA</u>**T

HindIII

3961 AAT**<u>AAGCTT</u>**G CATGCCTGCA GGTCGACTCT AGAGGATCCC CGGGTACCGA
GCTCGAATTC

SEQ ID NO 27

pMPX-63 (C-terminal fusion with Factor Xa TrxA residues 2-109 FLAG cloned into pMPX-5 using PCR-introduced PstI and BamHI)

Shine-Delgarno PstI
2401 GAATTCAGGCGCTTTTTAGACTGGTCGTAATGAAATTC**AGCAGG**ATCACATT**CTGCAG**AT
1 M

Factor Xa XbaI XhoI
2461 GATCGAAGCCCGC**TCTAGA***CTCGAG*AGCGATAAAATTATTCACCTGACTGACGACAGTTT
2 I E A R S R L E S D K I I H L T D D S F

2521 TGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTCGATTTCTGGGCAGAGTGGTG
22 D T D V L K A D G A I L V D F W A E W C

2581 CGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGCTGACGAATATCAGGGCAA
42 G P C K M I A P I L D E I A D E Y Q G K

2641 ACTGACCGTTGCAAAACTGAACATCGATCAAAACCCTGGCACTGCGCCGAAATATGGCAT
62 L T V A K L N I D Q N P G T A P K Y G I

2701 CCGTGGTATCCCGACTCTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGG
82 R G I P T L L L F K N G E V A A T K V G

XhoI
2761 TGCACTGTCTAAAGGTCAGTTGAAAGAGTTCCTCGACGCTAACCTGGCG**CTCGAG***GATTA*
102 A L S K G Q L K E F L D A N L A L E D Y

BamHI
2821 *TAAAGATCATGATGGCGATTATAAAGATCATGATGAT*TAATAA**GGATCC**CCGGGTACCGA
122 K D H D G D Y K D H D D

Gene *trxA* (2-109) from *E. coli* MG1655 cloned into pMPX-5. Create chimeric fusions with the *trxA* by cloning into PstI and XbaI. May remove *trxA* using XhoI. FLAG sequence shown in italics only.

SEQ ID NO:28

Rat Edg-3 nucleotide sequence

ATGGCAACCACGCACGCGCAGGGGCACCCGCCAGTCTTGGGGAATGATACTCTCCGGGAACATTATGATTACGTGGGGAAGCTGGCAGGCAGGCTGCGGGATCCCCCTGAGGGTAGCACCCTCATCACCACCATCCTCTTCTTGGTCACCTGTAGCTTCATCGTCTTGGAGAACCTGATGGTTTTGATTGCCATCTGGAAAAACAATAAATTTCATAACCGCATGTACTTTTTCATCGGCAACTTGGCTCTCTGCGACCTGCTGGCCGGCATAGCCTACAAGGTCAACATTCTGATGTCCGGTAGGAAGACGTTCAGCCTGTCTCCAACAGTGTGGTTCCTCAGGGAGGGCAGTATGTTCGTAGCCCTGGGCGCATCCACATGCAGCTTATTGGCCATTGCCATTGAGCGGCACCTGACCATGATCAAGATGAGGCCGTACGACGCCAACAAGAAGCACCGCGTGTTCCTTCTGATTGGGATGTGCTG

GCTAATTGCCTTCTCGCTGGGTGCCCTGCCCATCCTGGGCTGGAACTGCCTGGAGA
ACTTTCCCGACTGCTCTACCATCTTGCCCCTCTACTCCAAGAAATACATTGCCTTT
CTCATCAGCATCTTCACAGCCATTCTGGTGACCATCGTCATCTTGTACGCGCGCAT
CTACTTCCTGGTCAAGTCCAGCAGCCGCAGGGTGGCCAACCACAACTCCGAGAGA
TCCATGGCCCTTCTGCGGACCGTAGTGATCGTGGTGAGCGTGTTCATCGCCTGTTG
GTCCCCCCTTTTCATCCTCTTCCTCATCGATGTGGCCTGCAGGGCGAAGGAGTGCT
CCATCCTCTTCAAGAGTCAGTGGTTCATCATGCTGGCTGTCCTCAACTCGGCCATG
AACCCTGTCATCTACACGCTGGCCAGCAAAGAGATGCGGCGTGCTTTCTTCCGGTT
GGTGTGCGGCTGTCTGGTCAAGGGCAAGGGGACCCAGGCCTCCCCGATGCAGCCT
GCTCTTGACCCGAGCAGAAGTAAATCAAGCTCCAGTAACAACAGCAGCAGCCACT
CTCCAAAGGTCAAGGAAGACCTGCCCCATGTGGCTACCTCTTCCTGCGTCACTGA
CAAAACGAGGTCGCTTCAGAATGGGGTCCTCTGCAAGTGA -1145

SEQ ID NO:29

Rat Edg-3 amino acid sequence

M A T T H A Q G H P P V L G N D T L R E H Y D Y V G K L A G R L
R D P P E G S T L I T T I L F L V T C S F I V L E N L M V L I A
I W K N N K F H N R M Y F F I G N L A L C D L L A G I A Y K V N
I L M S G R K T F S L S P T V W F L R E G S M F V A L G A S T C
S L L A I A I E R H L T M I K M R P Y D A N K K H R V F L L I G
M C W L I A F S L G A L P I L G W N C L E N F P D C S T I L P L
Y S K K Y I A F L I S I F T A I L V T I V I L Y A R I Y F L V K
S S S R R V A N H N S E R S M A L L R T V V I V V S V F I A C W
S P L F I L F L I D V A C R A K E C S I L F K S Q W F I M L A V
L N S A M N P V I Y T L A S K E M R R A F F R L V C G C L V K G
K G T Q A S P M Q P A L D P S R S K S S S S N N S S S H S P K V
K E D L P H V A T S S C V T D K T R S L Q N G V L C K

******

SEQ ID NO.: 153

pMPX-66 arabinose-inducible expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT

HindIII

361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTCAAGCC GTCAATTGTC

Stop araC

421 TGATTCGTTA CCAATTATGA CAACTTGACG GCTACATCAT TCACTTTTTC TTCACAACCG

481 GCACGGAACT CGCTCGGGCT GGCCCCGGTG CATTTTTTAA ATACCCGCGA GAAATAGAGT

541 TGATCGTCAA AACCAACATT GCGACCGACG GTGGCGATAG GCATCCGGGT GGTGCTCAAA

601 AGCAGCTTCG CCTGGCTGAT ACGTTGGTCC TCGCGCCAGC TTAAGACGCT AATCCCTAAC

661 TGCTGGCGGA AAAGATGTGA CAGACGCGAC GGCGACAAGC AAACATGCTG TGCGACGCTG

721 GCGATATCAA AATTGCTGTC TGCCAGGTGA TCGCTGATGT ACTGACAAGC CTCGCGTACC

781 CGATTATCCA TCGGTGGATG GAGCGACTCG TTAATCGCTT CCATGCGCCG CAGTAACAAT

841 TGCTCAAGCA GATTTATCGC CAGCAGCTCC GAATAGCGCC CTTCCCCTTG CCCGGCGTTA

901 ATGATTTGCC CAAACAGGTC GCTGAAATGC GGCTGGTGCG CTTCATCCGG GCGAAAGAAC

961 CCCGTATTGG CAAATATTGA CGGCCAGTTA AGCCATTCAT GCCAGTAGGC GCGCGGACGA

1021 AAGTAAACCC ACTGGTGATA CCATTCGCGA GCCTCCGGAT GACGACCGTA GTGATGAATC

1081 TCTCCTGGCG GGAACAGCAA AATATCACCC GGTCGGCAAA CAAATTCTCG TCCCTGATTT

1141 TTCACCACCC CCTGACCGCG AATGGTGAGA TTGAGAATAT AACCTTTCAT TCCCAGCGGT

1201 CGGTCGATAA AAAAATCGAG ATAACCGTTG GCCTCAATCG GCGTTAAACC CGCCACCAGA

1261 TGGGCATTAA ACGAGTATCC CGGCAGCAGG GGATCATTTT GCGCTTCAGC CATACTTTTC

Start araC

1321 ATACTCCCGC CATTCAGAGA AGAAACCAAT TGTCCATATT GCATCAGACA TTGCCGTCAC

<--

1381 TGCGTCTTTT ACTGGCTCTT CTCGCTAACC AAACCGGTAA CCCCGCTTAT TAAAAGCATT

1441 CTGTAACAAA GCGGGACCAA AGCCATGACA AAAACGCGTA ACAAAAGTGT CTATAATCAC

1501 GGCAGAAAAG TCCACATTGA TTATTTGCAC GGCGTCACAC TTTGCTATGC CATAGCATTT

1561 TTATCCATAA GATTAGCGGA TCCTACCTGA CGCTTTTTAT CGCAACTCTC TACTGTTTCT

SD SalI XbaI

1621 CCATACCCGT TTTTTTGGGC TAGCAGGAGG CCGTCGACTC TAGAGGATCC CCGCGCCCTC

Stem-loop KpnI

1681 ATCCGAAAGG GCGTATTGGT ACCGAGCTCG AATTCGTAAT CATGGTCATA GCTGTTTCCT

1741 GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT

1801 AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC

1861 GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG

1921 AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG

1981 GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA

2041 GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC

2101 CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC

2161 AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

2221 TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC

2281 CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT

2341 CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG

2401 CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC

2461 TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

2521 GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT

2581 ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC

2641 AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA

2701 AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC

2761 GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC

2821 CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT

2881 GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA

2941 TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT

3001 GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA

3061 ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC

3121 ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG

3181 CGCAACGTTG TTGCCATTGC TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT

3241 TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA

3301 AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA

3361 TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC

3421 TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG AATAGTGTAT GCGGCGACCG

3481 AGTTGCTCTT GCCCGGCGTC AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA

3541 GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG

3601 AGATCCAGTT CGATGTAACC CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC

3661 ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG

3721 GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG AAGCATTTAT

3781 CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA

3841 GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCACCTGACG TCTAAGAAAC CATTATTATC

3901 ATGACATTAA CCTATAAAAA TAGGCGTATC ACGAGGCCCT TTCGTC

The segment araC through Para was taken from pBAD24 using PCR added HindIII and modified aligned Shine-Delgarno (SD) sequence with SalI followed by XbaI, a stem-loop transcriptional stop sequence, and KpnI. The PCR product was cloned into pUC18 using HindIII and KpnI.

SEQ ID NO.: 152

pMPX-72 rhamnose-inducible expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT

Stop rhaR

361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTAATTAA TCTTTCTGCG

HindIII

421 AATTGAGATG ACGCCACTGG CTGGGCGTCA TCCCGGTTTC CCGGGTAAAC ACCACCGAAA

481 AATAGTTACT ATCTTCAAAG CCACATTCGG TCGAAATATC ACTGATTAAC AGGCGGCTAT

541 GCTGGAGAAG ATATTGCGCA TGACACACTC TGACCTGTCG CAGATATTGA TTGATGGTCA

601 TTCCAGTCTG CTGGCGAAAT TGCTGACGCA AAACGCGCTC ACTGCACGAT GCCTCATCAC

661 AAAATTTATC CAGCGCAAAG GGACTTTTCA GGCTAGCCGC CAGCCGGGTA ATCAGCTTAT

721 CCAGCAACGT TTCGCTGGAT GTTGGCGGCA ACGAATCACT GGTGTAACGA TGGCGATTCA

781 GCAACATCAC CAACTGCCCG AACAGCAACT CAGCCATTTC GTTAGCAAAC GGCACATGCT

841 GACTACTTTC ATGCTCAAGC TGACCGATAA CCTGCCGCGC CTGCGCCATC CCCATGCTAC

901 CTAAGCGCCA GTGTGGTTGC CCTGCGCTGG CGTTAAATCC CGGAATCGCC CCCTGCCAGT

961 CAAGATTCAG CTTCAGACGC TCCGGGCAAT AAATAATATT CTGCAAAACC AGATCGTTAA

1021 CGGAAGCGTA GGAGTGTTTA TCGTCAGCAT GAATGTAAAA GAGATCGCCA CGGGTAATGC

1081 GATAAGGGCG ATCGTTGAGT ACATGCAGGC CATTACCGCG CCAGACAATC ACCAGCTCAC

1141 AAAAATCATG TGTATGTTCA GCAAAGACAT CTTGCGGATA ACGGTCAGCC ACAGCGACTG

1201 CCTGCTGGTC GCTGGCAAAA AAATCATCTT TGAGAAGTTT TAACTGATGC GCCACCGTGG

1261 CTACCTCGGC CAGAGAACGA AGTTGATTAT TCGCAATATG GCGTACAAAT ACGTTGAGAA

Stop rhaS Start rhaR

1321 GATTCGCGTT ATTGCAGAAA GCCATCCCGT CCCTGGCGAA TATCACGCGG TGACCAGTTA

<--

1381 AACTCTCGGC GAAAAAGCGT CGAAAAGTGG TTACTGTCGC TGAATCCACA GCGATAGGCG

1441 ATGTCAGTAA CGCTGGCCTC GCTGTGGCGT AGCAGATGTC GGGCTTTCAT CAGTCGCAGG

1501 CGGTTCAGGT ATCGCTGAGG CGTCAGTCCC GTTTGCTGCT TAAGCTGCCG ATGTAGCGTA

1561 CGCAGTGAAA GAGAAAATTG ATCCGCCACG GCATCCCAAT TCACCTCATC GGCAAAATGG

1621 TCCTCCAGCC AGGCCAGAAG CAAGTTGAGA CGTGATGCGC TGTTTTCCAG GTTCTCCTGC

1681 AAACTGCTTT TACGCAGCAA GAGCAGTAAT TGCATAAACA AGATCTCGCG ACTGGCGGTC

1741 GAGGGTAAAT CATTTTCCCC TTCCTGCTGT TCCATCTGTG CAACCAGCTG TCGCACCTGC

1801 TGCAATACGC TGTGGTTAAC GCGCCAGTGA GACGGATACT GCCCATCCAG CTCTTGTGGC

1861 AGCAACTGAT TCAGCCCGGC GAGAAACTGA AATCGATCCG GCGAGCGATA CAGCACATTG

1921 GTCAGACACA GATTATCGGT ATGTTCATAC AGATGCCGAT CATGATCGCG TACGAAACAG

1981 ACCGTGCCAC CGGTGATGGT ATAGGGCTGC CCATTAAACA CATGAATACC CGTGCCATGT

2041 TCGACAATCA CAATTTCATG AAAATCATGA TGATGTTCAG GAAAATCCGC CTGCGGGAGC

2101 CGGGGTTCTA TCGCCACGGA CGCGTTACCA GACGGAAAAA AATCCACACT ATGTAATACG

Start rhaS

2161 GTCATACTGG CCTCCTGATG TCGTCAACAC GGCGAAATAG TAATCACGAG GTCAGGTTCT

<--

2221 TACCTTAAAT TTTCGACGGA AAACCACGTA AAAAACGTCG ATTTTTCAAG ATACAGCGTG

2281 AATTTTCAGG AAATGCGGTG AGCATCACAT CACCACAATT CAGCAAATTG TGAACATCAT

2341 CACGTTCATC TTTCCCTGGT TGCCAATGGC CCATTTTCCT GTCAGTAACG AGAAGGTCGC

SD PstI SalI

2401 GAATTCAGGC GCTTTTTAGA CTGGTCGTAA TGAAATTCAG GAGGTTCTGC AGGTCGACTC

XbaI Stem-loop KpnI

2461 TAGAGGATCC CCGCGCCCTC ATCCGAAAGG GCGTATTGGT ACCGAGCTCG AATTCGTAAT

2521 CATGGTCATA GCTGTTTCCT GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC

2581 GAGCCGGAAG CATAAAGTGT AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA

2641 TTGCGTTGCG CTCACTGCCC GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT

2701 GAATCGGCCA ACGCGCGGGG AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC

2761 TCACTGACTC GCTGCGCTCG GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG

2821 CGGTAATACG GTTATCCACA GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG

2881 GCCAGCAAAA GGCCAGGAAC CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC

2941 GCCCCCCTGA CGAGCATCAC AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG

3001 GACTATAAAG ATACCAGGCG TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA

3061 CCCTGCCGCT TACCGGATAC CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC

3121 ATAGCTCACG CTGTAGGTAT CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG

3181 TGCACGAACC CCCCGTTCAG CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT

3241 CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA

3301 GAGCGAGGTA TGTAGGCGGT GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA

3361 CTAGAAGGAC AGTATTTGGT ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG

3421 TTGGTAGCTC TTGATCCGGC AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA

3481 AGCAGCAGAT TACGCGCAGA AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG

3541 GGTCTGACGC TCAGTGGAAC GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA

3601 AAAGGATCTT CACCTAGATC CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA

3661 TATATGAGTA AACTTGGTCT GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG

3721 CGATCTGTCT ATTTCGTTCA TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA

3781 TACGGGAGGG CTTACCATCT GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC

3841 CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC

3901 CTGCAACTTT ATCCGCCTCC ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA

3961 GTTCGCCAGT TAATAGTTTG CGCAACGTTG TTGCCATTGC TACAGGCATC GTGGTGTCAC

4021 GCTCGTCGTT TGGTATGGCT TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT

4081 GATCCCCCAT GTTGTGCAAA AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA

4141 GTAAGTTGGC CGCAGTGTTA TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG

4201 TCATGCCATC CGTAAGATGC TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG

4261 AATAGTGTAT GCGGCGACCG AGTTGCTCTT GCCCGGCGTC AATACGGGAT AATACCGCGC

4321 CACATAGCAG AACTTTAAAA GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT

4381 CAAGGATCTT ACCGCTGTTG AGATCCAGTT CGATGTAACC CACTCGTGCA CCCAACTGAT

4441 CTTCAGCATC TTTTACTTTC ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG

4501 CCGCAAAAAA GGGAATAAGG GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC

4561 AATATTATTG AAGCATTTAT CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA

4621 TTTAGAAAAA TAAACAAATA GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCACCTGACG

4681 TCTAAGAAAC CATTATTATC ATGACATTAA CCTATAAAAA TAGGCGTATC ACGAGGCCCT

4741 TTCGTC

The segment rhaR through Prha was taken from the E. coli chromosome using PCR added HindIII and modified aligned Shine-Delgarno (SD) sequence with PstI followed by SalI, XbaI, a stem-loop transcriptional stop sequence, and KpnI. The PCR product was cloned into pUC18 using HindIII and KpnI.

SEQ ID NO.: 151

pMPX-67 rhamnose-inducible expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT

Stop rhaR

361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTAATTAA TCTTTCTGCG

HindIII

421 AATTGAGATG ACGCCACTGG CTGGGCGTCA TCCCGGTTTC CCGGGTAAAC ACCACCGAAA

481 AATAGTTACT ATCTTCAAAG CCACATTCGG TCGAAATATC ACTGATTAAC AGGCGGCTAT

541 GCTGGAGAAG ATATTGCGCA TGACACACTC TGACCTGTCG CAGATATTGA TTGATGGTCA

601 TTCCAGTCTG CTGGCGAAAT TGCTGACGCA AAACGCGCTC ACTGCACGAT GCCTCATCAC

661 AAAATTTATC CAGCGCAAAG GGACTTTTCA GGCTAGCCGC CAGCCGGGTA ATCAGCTTAT

721 CCAGCAACGT TTCGCTGGAT GTTGGCGGCA ACGAATCACT GGTGTAACGA TGGCGATTCA

781 GCAACATCAC CAACTGCCCG AACAGCAACT CAGCCATTTC GTTAGCAAAC GGCACATGCT

841 GACTACTTTC ATGCTCAAGC TGACCGATAA CCTGCCGCGC CTGCGCCATC CCCATGCTAC

901 CTAAGCGCCA GTGTGGTTGC CCTGCGCTGG CGTTAAATCC CGGAATCGCC CCCTGCCAGT

961 CAAGATTCAG CTTCAGACGC TCCGGGCAAT AAATAATATT CTGCAAAACC AGATCGTTAA

1021 CGGAAGCGTA GGAGTGTTTA TCGTCAGCAT GAATGTAAAA GAGATCGCCA CGGGTAATGC

1081 GATAAGGGCG ATCGTTGAGT ACATGCAGGC CATTACCGCG CCAGACAATC ACCAGCTCAC

1141 AAAAATCATG TGTATGTTCA GCAAAGACAT CTTGCGGATA ACGGTCAGCC ACAGCGACTG

1201 CCTGCTGGTC GCTGGCAAAA AAATCATCTT TGAGAAGTTT TAACTGATGC GCCACCGTGG

1261 CTACCTCGGC CAGAGAACGA AGTTGATTAT TCGCAATATG GCGTACAAAT ACGTTGAGAA

Stop rhaS Start rhaR

1321 GATTCGCGTT ATTGCAGAAA GCCATCCCGT CCCTGGCGAA TATCACGCGG TGACCAGTTA

<--

1381 AACTCTCGGC GAAAAAGCGT CGAAAAGTGG TTACTGTCGC TGAATCCACA GCGATAGGCG

1441 ATGTCAGTAA CGCTGGCCTC GCTGTGGCGT AGCAGATGTC GGGCTTTCAT CAGTCGCAGG

1501 CGGTTCAGGT ATCGCTGAGG CGTCAGTCCC GTTTGCTGCT TAAGCTGCCG ATGTAGCGTA

1561 CGCAGTGAAA GAGAAAATTG ATCCGCCACG GCATCCCAAT TCACCTCATC GGCAAAATGG

1621 TCCTCCAGCC AGGCCAGAAG CAAGTTGAGA CGTGATGCGC TGTTTTCCAG GTTCTCCTGC

1681 AAACTGCTTT TACGCAGCAA GAGCAGTAAT TGCATAAACA AGATCTCGCG ACTGGCGGTC

1741 GAGGGTAAAT CATTTTCCCC TTCCTGCTGT TCCATCTGTG CAACCAGCTG TCGCACCTGC

1801 TGCAATACGC TGTGGTTAAC GCGCCAGTGA GACGGATACT GCCCATCCAG CTCTTGTGGC

1861 AGCAACTGAT TCAGCCCGGC GAGAAACTGA AATCGATCCG GCGAGCGATA CAGCACATTG

1921 GTCAGACACA GATTATCGGT ATGTTCATAC AGATGCCGAT CATGATCGCG TACGAAACAG

1981 ACCGTGCCAC CGGTGATGGT ATAGGGCTGC CCATTAAACA CATGAATACC CGTGCCATGT

2041 TCGACAATCA CAATTTCATG AAAATCATGA TGATGTTCAG GAAAATCCGC CTGCGGGAGC

2101 CGGGGTTCTA TCGCCACGGA CGCGTTACCA GACGGAAAAA AATCCACACT ATGTAATACG

Start rhaS

2161 GTCATACTGG CCTCCTGATG TCGTCAACAC GGCGAAATAG TAATCACGAG GTCAGGTTCT

<--

2221 TACCTTAAAT TTTCGACGGA AAACCACGTA AAAAACGTCG ATTTTTCAAG ATACAGCGTG

2281 AATTTTCAGG AAATGCGGTG AGCATCACAT CACCACAATT CAGCAAATTG TGAACATCAT

2341 CACGTTCATC TTTCCCTGGT TGCCAATGGC CCATTTTCCT GTCAGTAACG AGAAGGTCGC

SD SalI XbaI

2401 GAATTCAGGC GCTTTTTAGA CTGGTCGTAA TGAAATTCAG GAGGTTGTCG ACTCTAGAGG

Stem-loop KpnI

2461 ATCCCCGCGC CCTCATCCGA AAGGGCGTAT TGGTACCGAG CTCGAATTCG TAATCATGGT

2521 CATAGCTGTT TCCTGTGTGA AATTGTTATC CGCTCACAAT TCCACACAAC ATACGAGCCG

2581 GAAGCATAAA GTGTAAAGCC TGGGGTGCCT AATGAGTGAG CTAACTCACA TTAATTGCGT

2641 TGCGCTCACT GCCCGCTTTC CAGTCGGGAA ACCTGTCGTG CCAGCTGCAT TAATGAATCG

2701 GCCAACGCGC GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG

2761 ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA

2821 TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC

2881 AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC

2941 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT

3001 AAAGATACCA GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC

3061 CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT TCTCATAGCT

3121 CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG

3181 AACCCCCCGT TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC

3241 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA

3301 GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA

3361 GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA

3421 GCTCTTGATC CGGCAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC

3481 AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG

3541 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA

3601 TCTTCACCTA GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA AGTATATATG

3661 AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT

3721 GTCTATTTCG TTCATCCATA GTTGCCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG

3781 AGGGCTTACC ATCTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC

3841 CAGATTTATC AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA

3901 CTTTATCCGC CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC

3961 CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG TCACGCTCGT

4021 CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC

4081 CCATGTTGTG CAAAAAAGCG GTTAGCTCCT TCGGTCCTCC GATCGTTGTC AGAAGTAAGT

4141 TGGCCGCAGT GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC

4201 CATCCGTAAG ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT

4261 GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC GCGCCACATA

4321 GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA

4381 TCTTACCGCT GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG

4441 CATCTTTTAC TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA

4501 AAAAGGGAAT AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT

4561 ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA

4621 AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGTCTAAG

4681 AAACCATTAT TATCATGACA TTAACCTATA AAAATAGGCG TATCACGAGG CCCTTTCGTC

The segment rhaR through Prha was taken from the E. coli chromosome using PCR added HindIII and modified aligned Shine-Delgarno (SD) sequence with SalI followed by XbaI, a stem-loop transcriptional stop sequence, and KpnI. The PCR product was cloned into pUC18 using HindIII and KpnI.

SEQ ID NO.: 154

pMPX-71 arabinose-inducible expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT

HindIII

361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTCAAGCC GTCAATTGTC

Stop araC

421 TGATTCGTTA CCAATTATGA CAACTTGACG GCTACATCAT TCACTTTTTC TTCACAACCG

481 GCACGGAACT CGCTCGGGCT GGCCCCGGTG CATTTTTTAA ATACCCGCGA GAAATAGAGT

541 TGATCGTCAA AACCAACATT GCGACCGACG GTGGCGATAG GCATCCGGGT GGTGCTCAAA

601 AGCAGCTTCG CCTGGCTGAT ACGTTGGTCC TCGCGCCAGC TTAAGACGCT AATCCCTAAC

661 TGCTGGCGGA AAAGATGTGA CAGACGCGAC GGCGACAAGC AAACATGCTG TGCGACGCTG

721 GCGATATCAA AATTGCTGTC TGCCAGGTGA TCGCTGATGT ACTGACAAGC CTCGCGTACC

781 CGATTATCCA TCGGTGGATG GAGCGACTCG TTAATCGCTT CCATGCGCCG CAGTAACAAT

841 TGCTCAAGCA GATTTATCGC CAGCAGCTCC GAATAGCGCC CTTCCCCTTG CCCGGCGTTA

901 ATGATTTGCC CAAACAGGTC GCTGAAATGC GGCTGGTGCG CTTCATCCGG GCGAAAGAAC

961 CCCGTATTGG CAAATATTGA CGGCCAGTTA AGCCATTCAT GCCAGTAGGC GCGCGGACGA

1021 AAGTAAACCC ACTGGTGATA CCATTCGCGA GCCTCCGGAT GACGACCGTA GTGATGAATC

1081 TCTCCTGGCG GGAACAGCAA AATATCACCC GGTCGGCAAA CAAATTCTCG TCCCTGATTT

1141 TTCACCACCC CCTGACCGCG AATGGTGAGA TTGAGAATAT AACCTTTCAT TCCCAGCGGT

1201 CGGTCGATAA AAAAATCGAG ATAACCGTTG GCCTCAATCG GCGTTAAACC CGCCACCAGA

1261 TGGGCATTAA ACGAGTATCC CGGCAGCAGG GGATCATTTT GCGCTTCAGC CATACTTTTC

Start araC

1321 ATACTCCCGC CATTCAGAGA AGAAACCAAT TGTCCATATT GCATCAGACA TTGCCGTCAC

<--

1381 TGCGTCTTTT ACTGGCTCTT CTCGCTAACC AAACCGGTAA CCCCGCTTAT TAAAAGCATT

1441 CTGTAACAAA GCGGGACCAA AGCCATGACA AAAACGCGTA ACAAAAGTGT CTATAATCAC

1501 GGCAGAAAAG TCCACATTGA TTATTTGCAC GGCGTCACAC TTTGCTATGC CATAGCATTT

1561 TTATCCATAA GATTAGCGGA TCCTACCTGA CGCTTTTTAT CGCAACTCTC TACTGTTTCT

SD PstI SalI XbaI

1621 CCATACCCGT TTTTTTGGGC TAGCAGGAGG CCCTGCAGGT CGACTCTAGA GGATCCCCGC

Stem-loop KpnI

1681 GCCCTCATCC GAAAGGGCGT ATTGGTACCG AGCTCGAATT CGTAATCATG GTCATAGCTG

1741 TTTCCTGTGT GAAATTGTTA TCCGCTCACA ATTCCACACA ACATACGAGC CGGAAGCATA

1801 AAGTGTAAAG CCTGGGGTGC CTAATGAGTG AGCTAACTCA CATTAATTGC GTTGCGCTCA

1861 CTGCCCGCTT TCCAGTCGGG AAACCTGTCG TGCCAGCTGC ATTAATGAAT CGGCCAACGC

1921 GCGGGGAGAG GCGGTTTGCG TATTGGGCGC TCTTCCGCTT CCTCGCTCAC TGACTCGCTG

1981 CGCTCGGTCG TTCGGCTGCG GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA

2041 TCCACAGAAT CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC

2101 AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC CCCTGACGAG

2161 CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC

2221 CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC

2281 GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT

2341 AGGTATCTCA GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC

2401 GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA

2461 CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA TTAGCAGAGC GAGGTATGTA

2521 GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG CCTAACTACG GCTACACTAG AAGGACAGTA

2581 TTTGGTATCT GCGCTCTGCT GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA

2641 TCCGGCAAAC AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG

2701 CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC TGACGCTCAG

2761 TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT TATCAAAAAG GATCTTCACC

2821 TAGATCCTTT TAAATTAAAA ATGAAGTTTT AAATCAATCT AAAGTATATA TGAGTAAACT

2881 TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT

2941 CGTTCATCCA TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA

3001 CCATCTGGCC CCAGTGCTGC AATGATACCG CGAGACCCAC GCTCACCGGC TCCAGATTTA

3061 TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC

3121 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT

3181 AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT

3241 ATGGCTTCAT TCAGCTCCGG TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG

3301 TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA GTTGGCCGCA

3361 GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA

3421 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA GTGTATGCGG

3481 CGACCGAGTT GCTCTTGCCC GGCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT

3541 TTAAAAGTGC TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG

3601 CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT

3661 ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA

3721 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTTCAATA TTATTGAAGC

3781 ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA

3841 CAAATAGGGG TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT

3901 ATTATCATGA CATTAACCTA TAAAAATAGG CGTATCACGA GGCCCTTTCG TC

The segment araC through Para was taken from pBAD24 using PCR added HindIII and modified aligned Shine-Delgarno (SD) sequence with PstI followed by SalI, XbaI, a stem-loop transcriptional stop sequence, and KpnI. The PCR product was cloned into pUC18 using HindIII and KpnI.

SEQ ID NO.: 155

pMPX-68 melibiose-inducible expression vector

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT

301 TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT

HindIII

361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTTTAGCC GGGAAACGTC

Stop MelR

421 TGGCGGCGCT GTTGGCTAAG TTTGCGGTAT TGTTGCGGCG ACATGCCGAC ATATTTGCCG

481 AACGTGCTGT AAAAACGACT ACTTGAACGA AAGCCTGCCG TCAGGGCAAT ATCGAGAATA

541 CTTTTATCGG TATCGCTCAG TAACGCGCGA ACGTGGTTGA TGCGCATCGC GGTAATGTAC

601 TGTTTCATCG TCAATTGCAT GACCCGCTGG AATATCCCCA TTGCATAGTT GGCGTTAAGT

661 TTGACGTGCT CAGCCACATC GTTGATGGTC AGCGCCTGAT CATAGTTTTC GGCAATAAAG

721 CCCAGCATCT GGCTAACATA AAATTGCGCA TGGCGCGAGA CGCTGTTTTT GTGTGTGCGC

781 GAGGTTTTAT TGACCAGAAT CGGTTCCCAG CCAGAGAGGC TAAATCGCTT GAGCATCAGG

841 CCAATTTCAT CAATGGCGAG CTGGCGAATT TGCTCGTTCG GACTGTTTAA TTCCTGCTGC

901 CAGCGGCGCA CTTCAAACGG GCTAAGTTGC TGTGTGGCCA GTGATTTGAT CACCATGCCG

961 TGAGTGACGT GGTTAATCAG GTCTTTATCC AGCGGCCAGG AGAGAAACAG ATGCATCGGC

1021 AGATTAAAAA TCGCCATGCT CTGACAGGTT CCGGTATCTG TTAGTTGGTG CGGTGTACAG

1081 GCCCAGAACA GCGTGATATG ACCCTGATTG ATATTCACTT TTTCATTGTT GATCAGGTAT

1141 TCCACATCGC CATCGAAAGG CACATTCACT TCGACCTGAC CATGCCAGTG GCTGGTGGGC

1201 ATGATATGCG GTGCGCGAAA CTCAATCTCC ATCCGCTGGT ATTCCGAATA CAGCGACAGC

+1

MelR

1261 GGGCTGCGGG TCTGTTTTTC GTCGCTGCTG CACATAAACG TATCTGTATT CATGGATGGC

1321 TCTCTTTCCT GGAATATCAG AATTATGGCA GGAGTGAGGG AGGATGACTG CGAGTGGGAG

1381 CACGGTTTTC ACCCTCTTCC CAGAGGGGCG AGGGGACTCT CCGAGTATCA TGAGGCCGAA

1441 AACTCTGCTT TTCAGGTAAT TTATTCCCAT AAACTCAGAT TTACTGCTGC TTCACGCAGG

1501 ATCTGAGTTT ATGGGAATGC TCAACCTGGA AGCCGGAGGT TTTCTGCAGA TTCGCCTGCC

SD SalI XbaI

1561 ATGATGAAGT TATTCAAGCA AGCCAGGAGG TCGTCGACTC TAGAGGATCC CCGCGCCCTC

Stem-loop KpnI

1621 ATCCGAAAGG GCGTATTGGT ACCGAGCTCG AATTCGTAAT CATGGTCATA GCTGTTTCCT

1681 GTGTGAAATT GTTATCCGCT CACAATTCCA CACAACATAC GAGCCGGAAG CATAAAGTGT

1741 AAAGCCTGGG GTGCCTAATG AGTGAGCTAA CTCACATTAA TTGCGTTGCG CTCACTGCCC

1801 GCTTTCCAGT CGGGAAACCT GTCGTGCCAG CTGCATTAAT GAATCGGCCA ACGCGCGGGG

1861 AGAGGCGGTT TGCGTATTGG GCGCTCTTCC GCTTCCTCGC TCACTGACTC GCTGCGCTCG

1921 GTCGTTCGGC TGCGGCGAGC GGTATCAGCT CACTCAAAGG CGGTAATACG GTTATCCACA

1981 GAATCAGGGG ATAACGCAGG AAAGAACATG TGAGCAAAAG GCCAGCAAAA GGCCAGGAAC

2041 CGTAAAAAGG CCGCGTTGCT GGCGTTTTTC CATAGGCTCC GCCCCCCTGA CGAGCATCAC

2101 AAAAATCGAC GCTCAAGTCA GAGGTGGCGA AACCCGACAG GACTATAAAG ATACCAGGCG

2161 TTTCCCCCTG GAAGCTCCCT CGTGCGCTCT CCTGTTCCGA CCCTGCCGCT TACCGGATAC

2221 CTGTCCGCCT TTCTCCCTTC GGGAAGCGTG GCGCTTTCTC ATAGCTCACG CTGTAGGTAT

2281 CTCAGTTCGG TGTAGGTCGT TCGCTCCAAG CTGGGCTGTG TGCACGAACC CCCCGTTCAG

2341 CCCGACCGCT GCGCCTTATC CGGTAACTAT CGTCTTGAGT CCAACCCGGT AAGACACGAC

2401 TTATCGCCAC TGGCAGCAGC CACTGGTAAC AGGATTAGCA GAGCGAGGTA TGTAGGCGGT

2461 GCTACAGAGT TCTTGAAGTG GTGGCCTAAC TACGGCTACA CTAGAAGGAC AGTATTTGGT

2521 ATCTGCGCTC TGCTGAAGCC AGTTACCTTC GGAAAAAGAG TTGGTAGCTC TTGATCCGGC

2581 AAACAAACCA CCGCTGGTAG CGGTGGTTTT TTTGTTTGCA AGCAGCAGAT TACGCGCAGA

2641 AAAAAAGGAT CTCAAGAAGA TCCTTTGATC TTTTCTACGG GGTCTGACGC TCAGTGGAAC

2701 GAAAACTCAC GTTAAGGGAT TTTGGTCATG AGATTATCAA AAAGGATCTT CACCTAGATC

2761 CTTTTAAATT AAAAATGAAG TTTTAAATCA ATCTAAAGTA TATATGAGTA AACTTGGTCT

2821 GACAGTTACC AATGCTTAAT CAGTGAGGCA CCTATCTCAG CGATCTGTCT ATTTCGTTCA

2881 TCCATAGTTG CCTGACTCCC CGTCGTGTAG ATAACTACGA TACGGGAGGG CTTACCATCT

2941 GGCCCCAGTG CTGCAATGAT ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA

3001 ATAAACCAGC CAGCCGGAAG GGCCGAGCGC AGAAGTGGTC CTGCAACTTT ATCCGCCTCC

3061 ATCCAGTCTA TTAATTGTTG CCGGGAAGCT AGAGTAAGTA GTTCGCCAGT TAATAGTTTG

3121 CGCAACGTTG TTGCCATTGC TACAGGCATC GTGGTGTCAC GCTCGTCGTT TGGTATGGCT

3181 TCATTCAGCT CCGGTTCCCA ACGATCAAGG CGAGTTACAT GATCCCCCAT GTTGTGCAAA

3241 AAAGCGGTTA GCTCCTTCGG TCCTCCGATC GTTGTCAGAA GTAAGTTGGC CGCAGTGTTA

3301 TCACTCATGG TTATGGCAGC ACTGCATAAT TCTCTTACTG TCATGCCATC CGTAAGATGC

3361 TTTTCTGTGA CTGGTGAGTA CTCAACCAAG TCATTCTGAG AATAGTGTAT GCGGCGACCG

3421 AGTTGCTCTT GCCCGGCGTC AATACGGGAT AATACCGCGC CACATAGCAG AACTTTAAAA

3481 GTGCTCATCA TTGGAAAACG TTCTTCGGGG CGAAAACTCT CAAGGATCTT ACCGCTGTTG

3541 AGATCCAGTT CGATGTAACC CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC

3601 ACCAGCGTTT CTGGGTGAGC AAAAACAGGA AGGCAAAATG CCGCAAAAAA GGGAATAAGG

3661 GCGACACGGA AATGTTGAAT ACTCATACTC TTCCTTTTTC AATATTATTG AAGCATTTAT

3721 CAGGGTTATT GTCTCATGAG CGGATACATA TTTGAATGTA TTTAGAAAAA TAAACAAATA

3781 GGGGTTCCGC GCACATTTCC CCGAAAAGTG CCACCTGACG TCTAAGAAAC CATTATTATC

3841 ATGACATTAA CCTATAAAAA TAGGCGTATC ACGAGGCCCT TTCGTC

SEQ ID NO.: 166

MalE (1-370) Factor Xa NTR (43-424) FLAG

SalI +1 MalE (1-370)

1

GTCGACATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAACGA CGATGATGTTT

1 M K I K T G A R I L A L S A L T T M M F

61

TCCGCCTCGGCTCTCGCCAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACG GCGAT

21 S A S A L A K I E E G K L V I W I N G D

121

AAAGGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAA TTAAA

41 K G Y N G L A E V G K K F E K D T G I K

181

GTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTCCCACAGGTTGCGGCAA CTGGC

61 V T V E H P D K L E E K F P Q V A A T G

241
GATGGCCCTGACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATC
TGGC

81 D G P D I I F W A H D R F G G Y A Q S G

301
CTGTTGGCTGAAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTA
CCTGG

101 L L A E I T P D K A F Q D K L Y P F T W

361
GATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTT
ATCG

121 D A V R Y N G K L I A Y P I A V E A L S

421
CTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCC
CGGCG

141 L I Y N K D L L P N P P K T W E E I P A

481
CTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAG
AACCG

161 L D K E L K A K G K S A L M F N L Q E P

541
TACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGC

181 Y F T W P L I A A D G G Y A F K Y E N G

601
AAGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGACCTTC

201 K Y D I K D V G V D N A G A K A G L T F

661
CTGGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCGCAGAA

221 L V D L I K N K H M N A D T D Y S I A E

721
GCTGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGTCCAAC

241 A A F N K G E T A M T I N G P W A W S N

781
ATCGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAGGGTCAACCA

261 I D T S K V N Y G V T V L P T F K G Q P

841
TCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCGAACA AAGAG

281 S K P F V G V L S A G I N A A S P N K E

901
CTGGCGAAAGAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGGAAGCGG TTAAT

301 L A K E F L E N Y L L T D E G L E A V N

961
AAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGAGTTGGCGA AAGAT

321 K D K P L G A V A L K S Y E E E L A K D

1021
CCACGTATTGCCGCCACCATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACA TCCCG

341 P R I A A T M E N A Q K G E I M P N I P

Factor Xa +43 NTR

1081
CAGATGTCCGCTTTCTGGTATGCCGTGCTGATCGAAGCCCGCACCTCGGAATCCGA CACG

361 Q M S A F W Y A V L I E A R T S E S D T

1141
GCAGGGCCCAACAGCGACCTGGACGTGAACACTGACATTTATTCCAAGGTGCTGG
TGACT

381 A G P N S D L D V N T D I Y S K V L V T

1201
GCTATATACCTGGCACTCTTCGTGGTGGGCACTGTGGGCAACTCCGTGACAGCCTT
CACT

401 A I Y L A L F V V G T V G N S V T A F T

1261
CTAGCGCGGAAGAAGTCACTGCAGAGCCTGCAGAGCACTGTGCATTACCACCTGG
GCAGC

421 L A R K K S L Q S L Q S T V H Y H L G S

1321
CTGGCACTGTCGGACCTGCTTATCCTTCTGCTGGCCATGCCCGTGGAGCTATACAA
CTTC

441 L A L S D L L I L L L A M P V E L Y N F

1381
ATCTGGGTACACCATCCCTGGGCCTTTGGGGACGCTGGCTGCCGTGGCTACTATTT
CCTG

461 I W V H H P W A F G D A G C R G Y Y F L

1441
CGTGATGCCTGCACCTATGCCACAGCCCTCAATGTAGCCAGCCTGAGTGTGGAGC
GCTAC

481 R D A C T Y A T A L N V A S L S V E R Y

1501
TTGGCCATCTGCCATCCCTTCAAGGCCAAGACCCTCATGTCCCGCAGCCGCACCA
AGAAA

501 L A I C H P F K A K T L M S R S R T K K

1561
TTCATCAGTGCCATATGGCTAGCTTCGGCGCTGCTGGCTATACCCATGCTTTTCAC
CATG

521 F I S A I W L A S A L L A I P M L F T M

1621
GGCCTGCAGAACCGCAGTGGTGACGGCACGCACCCTGGCGGCCTGGTGTGCACAC
CCATT

541 G L Q N R S G D G T H P G G L V C T P I

1681
GTGGACACAGCCACTGTCAAGGTCGTCATCCAGGTTAACACCTTCATGTCCTTCCT
GTTT

561 V D T A T V K V V I Q V N T F M S F L F

1741

CCCATGTTGGTCATCTCCATCCTAAACACCGTGATTGCCAACAAACTGACAGTCATGGTG

581 P M L V I S I L N T V I A N K L T V M V

1801

CACCAGGCCGCCGAGCAGGGCCGAGTGTGCACCGTGGGCACACACAACGGTTTAGAGCAC

601 H Q A A E Q G R V C T V G T H N G L E H

1861

AGCACGTTCAACATGACCATCGAGCCGGGTCGTGTCCAGGCCCTGCGCCACGGAGTCCTC

621 S T F N M T I E P G R V Q A L R H G V L

1921

GTCTTACGTGCTGTGGTCATTGCCTTTGTGGTCTGCTGGCTGCCCTACCACGTGCGACGC

641 V L R A V V I A F V V C W L P Y H V R R

1981

CTGATGTTCTGCTATATCTCGGATGAACAGTGGACTACGTTCCTCTTCGATTTCTACCAC

661 L M F C Y I S D E Q W T T F L F D F Y H

2041
TATTTCTACATGCTAACCAACGCTCTCTTCTACGTCAGCTCCGCCATCAATCCCATCCTC

681 Y F Y M L T N A L F Y V S S A I N P I L

2101
TACAACCTGGTCTCCGCCAACTTCCGCCAGGTCTTTCTGTCCACGCTGGCCTGCCTTTGT

701 Y N L V S A N F R Q V F L S T L A C L C

2161
CCTGGGTGGCGCCACCGCCGAAAGAAGAGGCCAACGTTCTCCAGGAAGCCCAACAGCATG

721 P G W R H R R K K R P T F S R K P N S M

NotI

2221
TCCAGCAACCATGCCTTTTCCACCAGCGCCACCCGGGAGACCCTGTACgcggccgca

741 S S N H A F S T S A T R E T L Y A A A

Flag stop KpnI

GATTATAAAGATGACGATGACAAATAATAAGGTACC

D Y K D D D D K * *

SEQ ID NO.: 167

MalE (1-28) Factor Xa NTR (43-424) FLAG

SalI +1 MalE leader (1-28)

1

gtcgacATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAACGACG ATGATGTTT

1 M K I K T G A R I L A L S A L T T M M F

Factor Xa +43 NTR

61

TCCGCCTCGGCTCTCGCCAAAATCATCGAAGCCCGCACCTCGGAATCCGACACGG CAGGG

21 S A S A L A K I I E A R T S E S D T A G

121

CCCAACAGCGACCTGGACGTGAACACTGACATTTATTCCAAGGTGCTGGTGACTG CTATA

41 P N S D L D V N T D I Y S K V L V T A I

181
TACCTGGCACTCTTCGTGGTGGGCACTGTGGGCAACTCCGTGACAGCCTTCACTCT
AGCG

61 Y L A L F V V G T V G N S V T A F T L A

241
CGGAAGAAGTCACTGCAGAGCCTGCAGAGCACTGTGCATTACCACCTGGGCAGCC
TGGCA

81 R K K S L Q S L Q S T V H Y H L G S L A

301
CTGTCGGACCTGCTTATCCTTCTGCTGGCCATGCCCGTGGAGCTATACAACTTCAT
CTGG

101 L S D L L I L L L A M P V E L Y N F I W

361
GTACACCATCCCTGGGCCTTTGGGGACGCTGGCTGCCGTGGCTACTATTTCCTGCG
TGAT

121 V H H P W A F G D A G C R G Y Y F L R D

421
GCCTGCACCTATGCCACAGCCCTCAATGTAGCCAGCCTGAGTGTGGAGCGCTACT
TGGCC

141 A C T Y A T A L N V A S L S V E R Y L A

481
ATCTGCCATCCCTTCAAGGCCAAGACCCTCATGTCCCGCAGCCGCACCAAGAAAT
TCATC

161 I C H P F K A K T L M S R S R T K K F I

541
AGTGCCATATGGCTAGCTTCGGCGCTGCTGGCTATACCCATGCTTTTCACCATGGG
CCTG

181 S A I W L A S A L L A I P M L F T M G L

601
CAGAACCGCAGTGGTGACGGCACGCACCCTGGCGGCCTGGTGTGCACACCCATTG
TGGAC

201 Q N R S G D G T H P G G L V C T P I V D

661
ACAGCCACTGTCAAGGTCGTCATCCAGGTTAACACCTTCATGTCCTTCCTGTTTCC
CATG

221 T A T V K V V I Q V N T F M S F L F P M

721
TTGGTCATCTCCATCCTAAACACCGTGATTGCCAACAAACTGACAGTCATGGTGCA
CCAG

241 L V I S I L N T V I A N K L T V M V H Q

781
GCCGCCGAGCAGGGCCGAGTGTGCACCGTGGGCACACACAACGGTTTAGAGCACA
GCACG

261 A A E Q G R V C T V G T H N G L E H S T

841
TTCAACATGACCATCGAGCCGGGTCGTGTCCAGGCCCTGCGCCACGGAGTCCTCG
TCTTA

281 F N M T I E P G R V Q A L R H G V L V L

901
CGTGCTGTGGTCATTGCCTTTGTGGTCTGCTGGCTGCCCTACCACGTGCGACGCCT
GATG

301 R A V V I A F V V C W L P Y H V R R L M

961
TTCTGCTATATCTCGGATGAACAGTGGACTACGTTCCTCTTCGATTTCTACCACTA
TTTC

321 F C Y I S D E Q W T T F L F D F Y H Y F

1021
TACATGCTAACCAACGCTCTCTTCTACGTCAGCTCCGCCATCAATCCCATCCTCTA
CAAC

341 Y M L T N A L F Y V S S A I N P I L Y N

1081

CTGGTCTCCGCCAACTTCCGCCAGGTCTTTCTGTCCACGCTGGCCTGCCTTTGTCC TGGG

361 L V S A N F R Q V F L S T L A C L C P G

1141

TGGCGCCACCGCCGAAAGAAGAGGCCAACGTTCTCCAGGAAGCCCAACAGCATGT CCAGC

381 W R H R R K K R P T F S R K P N S M S S

NotI Flag

1201

AACCATGCCTTTTCCACCAGCGCCACCCGGGAGACCCTGTACgcggccgcaGATTATA AA

401 N H A F S T S A T R E T L Y A A A D Y K

stop KpnI

GATGACGATGACAAATAATAAGGTACC

D D D D K

SEQ ID NO.: 169

MalE (1-370) Factor Xa NTR (43-424) TrxA (2-109) FLAG

SalI +1 MalE (1-370)

1

GTCGACATGAAAATAAAAACAGGTGCACGCATCCTCGCATTATCCGCATTAACGACGATGATGTTT

1 M K I K T G A R I L A L S A L T T M M F

61

TCCGCCTCGGCTCTCGCCAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGAT

21 S A S A L A K I E E G K L V I W I N G D

121

AAAGGCTATAACGGTCTCGCTGAAGTCGGTAAGAAATTCGAGAAAGATACCGGAATTAAA

41 K G Y N G L A E V G K K F E K D T G I K

181

GTCACCGTTGAGCATCCGGATAAACTGGAAGAGAAATTCCCACAGGTTGCGGCAACTGGC

61 V T V E H P D K L E E K F P Q V A A T G

241

GATGGCCCTGACATTATCTTCTGGGCACACGACCGCTTTGGTGGCTACGCTCAATCTGGC

81 D G P D I I F W A H D R F G G Y A Q S G

301

CTGTTGGCTGAAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTACCTGG

101 L L A E I T P D K A F Q D K L Y P F T W

361

GATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCG

121 D A V R Y N G K L I A Y P I A V E A L S

421

CTGATTTATAACAAAGATCTGCTGCCGAACCCGCCAAAAACCTGGGAAGAGATCCCGGCG

141 L I Y N K D L L P N P P K T W E E I P A

481

CTGGATAAAGAACTGAAAGCGAAAGGTAAGAGCGCGCTGATGTTCAACCTGCAAGAACCG

161 L D K E L K A K G K S A L M F N L Q E P

541

TACTTCACCTGGCCGCTGATTGCTGCTGACGGGGGTTATGCGTTCAAGTATGAAAACGGC

181 Y F T W P L I A A D G G Y A F K Y E N G

601

AAGTACGACATTAAAGACGTGGGCGTGGATAACGCTGGCGCGAAAGCGGGTCTGA CCTTC

201 K Y D I K D V G V D N A G A K A G L T F

661

CTGGTTGACCTGATTAAAAACAAACACATGAATGCAGACACCGATTACTCCATCG CAGAA

221 L V D L I K N K H M N A D T D Y S I A E

721

GCTGCCTTTAATAAAGGCGAAACAGCGATGACCATCAACGGCCCGTGGGCATGGT CCAAC

241 A A F N K G E T A M T I N G P W A W S N

781

ATCGACACCAGCAAAGTGAATTATGGTGTAACGGTACTGCCGACCTTCAAGGGTC AACCA

261 I D T S K V N Y G V T V L P T F K G Q P

841

TCCAAACCGTTCGTTGGCGTGCTGAGCGCAGGTATTAACGCCGCCAGTCCGAACA AAGAG

281 S K P F V G V L S A G I N A A S P N K E

901

CTGGCGAAAGAGTTCCTCGAAAACTATCTGCTGACTGATGAAGGTCTGGAAGCGG TTAAT

301 L A K E F L E N Y L L T D E G L E A V N

961

AAAGACAAACCGCTGGGTGCCGTAGCGCTGAAGTCTTACGAGGAAGAGTTGGCGA AAGAT

321 K D K P L G A V A L K S Y E E E L A K D

1021

CCACGTATTGCCGCCACCATGGAAAACGCCCAGAAAGGTGAAATCATGCCGAACA TCCCG

341 P R I A A T M E N A Q K G E I M P N I P

Factor Xa +43 NTR

1081

CAGATGTCCGCTTTCTGGTATGCCGTGCTGATCGAAGCCCGCACCTCGGAATCCGA CACG

361 Q M S A F W Y A V L I E A R T S E S D T

1141
GCAGGGCCCAACAGCGACCTGGACGTGAACACTGACATTTATTCCAAGGTGCTGG
TGACT

381 A G P N S D L D V N T D I Y S K V L V T

1201
GCTATATACCTGGCACTCTTCGTGGTGGGCACTGTGGGCAACTCCGTGACAGCCTT
CACT

401 A I Y L A L F V V G T V G N S V T A F T

1261
CTAGCGCGGAAGAAGTCACTGCAGAGCCTGCAGAGCACTGTGCATTACCACCTGG
GCAGC

421 L A R K K S L Q S L Q S T V H Y H L G S

1321
CTGGCACTGTCGGACCTGCTTATCCTTCTGCTGGCCATGCCCGTGGAGCTATACAA
CTTC

441 L A L S D L L I L L L A M P V E L Y N F

1381
ATCTGGGTACACCATCCCTGGGCCTTTGGGGACGCTGGCTGCCGTGGCTACTATTT
CCTG

461 I W V H H P W A F G D A G C R G Y Y F L